HEAT STRESS RESISTANCE IN PEA (*Pisum sativum* L.) BASED ON CANOPY AND LEAF TRAITS

A Thesis Submitted to the College of Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon, Saskatchewan

By Endale Geta Tafesse

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor who supervised my thesis/dissertation work or, in her absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

Request for permission to copy or to make other use of material in this thesis, in whole or part, should be addressed to:

Head of the Department of Plant Sciences
51 Campus Drive
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5A8 Canada

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9
Canada

ABSTRACT

Pea (*Pisum sativum* L.) is a grain legume widely grown in semi-arid conditions in western Canada, where summer daily temperatures may rise above 28 °C for several days, hampering plant growth and yield. Heat stress leads to impaired photosynthesis, shortened life cycle, abortion of flowers, pods and ovules, and thus yield loss. To minimize yield loss and stabilize production, heat resistance is a desirable trait. Pea has genetically diverse leaf and canopy characteristics including plant growth habit, leaf type, determinacy, canopy color, flower color, leaf wax and more that may be used in searching for traits to mitigate heat stress. The goals of this study were to identify leaf, canopy and biochemical traits involved in pea heat resistance, and determine effects of individual or combined heat and drought stresses on physiological, growth and yield performance.

Results from field trials across six environments in western Canada using 24 cultivars demonstrated that heat stress increased canopy temperature (CT), leaf chlorophyll a/b ratio, leaf wax and leaf anthocyanin concentrations, but reduced leaf chlorophyll a, chlorophyll b, and carotenoid concentrations, plant height, reproductive stem length, internode length, flowering duration, pod number, pod set ratio, and seed yield. The pea cultivars had a differential response to the various growth and yield traits. Under heat stress environments, cultivars with the semileafless leaf type and upright canopy habit had a significantly lower CT (up to 2 °C), and greater heat tolerance index (HTI) than cultivars with the vining habit and normal leaf type, likely due to less ground contact, high light reflection in the visible and near infrared wavelength and enhanced aeration through the canopy. Lodging contributed to high CT and exacerbated heat susceptibility. In contrast, under non-heat stress conditions, cultivars with the normal leaf type, indeterminate and vining canopy habit had greater yield potential.

Generally, greater leaf pigment and wax concentrations were associated with high HTI and contributed to a lower CT in the field. Vegetative indices including photochemical reflectance index (PRI), green normalized vegetation index (GNDVI), normalized pigments and chlorophyll index (NPCI), and the water band index (WBI) showed a consistent relationship with heat tolerance traits. Exogenous wax application of 100 µg stipule^{-1} on selected cultivars under the field condition led to 40 and 14% more radiation reflected in the ultraviolet (UV) and near infrared (NIR) regions, respectively compared to an untreated control. Enhanced reflection in the UV and NIR regions was associated with excess heat avoidance. Shading resulted in a significant chlorophyll and carotenoid loss, which led to enhanced spectral reflectance in the visible spectral

regions. Limited light absorption and thus photosynthesis efficiency was demonstrated by a low (63% less) PRI and a high (over 700%) NPCI compared to the control. As a heat avoidance trait, leaf surface wax concentration reduced organ heat absorption by enhanced reflection both in the high energy UV and in the NIR regions.

A controlled growth chamber experiment to examine individual and combined effects of heat and drought demonstrated that stomatal conductance and cumulative evapotranspiration decreased due to drought and the combined stresses of heat and drought, but not by heat stress alone. Under the heat treatment, optimal water supply reduced leaf temperature by 2.2 °C. Pea growth and seed yield traits decreased due to heat or drought, and their combined occurrence exacerbated their individual impacts. Drought and combined stress effects had a similar pattern although the combined stress was most detrimental onto overall pea performance. Pea cultivars differed in sensitivity to drought and heat stresses.

The second controlled growth chamber experiment had a 5 to 7 °C greater threshold temperature compared to the field for comparable growth and yield damage. Growth related parameters including plant height, internode length, and node numbers, had high thresholds ($\geq$ 34 °C) for significant growth reduction whereas yield related traits including pod numbers and seed yield had relatively low ($\leq$ 31 °C) thresholds for yield loss. Expanding leaves were more sensitive to heat stress and had a lower threshold temperature than mature and senescing leaves.

Overall, cultivars with the semileafless leaf type and upright nature were better adapted to heat and drought stressed environments than cultivars with the normal leaf and vining habit, and maintained a cooler CT and overall greater yield. Leaf spectral reflectance was dependent on pigments, wax, and leaf water content. The possibility of applying exogenous wax to leaf surfaces to augment naturally existing wax content to enhance the plants' heat avoidance capacity was novel. Canopy temperature and VIs including NDVI, PRI, NPCI and WBI can be used to indicate the overall physiological and biochemical status of a plant. Finally, optimal soil water supply can moderate the impacts of heat stress by 2 °C.

ACKNOWLEDGEMENTS

I am grateful to my supervisor Dr. Rosalind Bueckert for giving me the opportunity to conduct my PhD study under her supervision. Dr. Bueckert provided continuous guidance, expertise, and encouragement throughout my study. My appreciation extends to my advisory committee members, Dr. Thomas Warkentin, Dr. Steve Shirtliffe, Dr. Kirstin Bett, Dr. Scott Noble, and Dr. Yuguang Bai, for their support and valuable advice on my thesis work. A special appreciation goes to Dr. Warkentin; I could not have completed my project if it were not for his additional guidance, motivation and financial support. I am grateful to summer students B. Louie, J. Denis, Z. Wang, S. Ryu, R. Xiang, D. Maclean, and P. Bangar for their technical assistance during the lab and field measurements. I also thank the pulse crops technicians and field lab members for field maintenance.

This work was financially supported by the Saskatchewan Agriculture Development Fund, Saskatchewan Pulse Crop Development Board, and Western Grains Research Foundation. Also, I am grateful for the various scholarships I received, including the Saskatchewan Innovation and Opportunity Scholarship, 2017/18, Alexander and Jean Auckland Postgraduate Bursary 2016/17, Rene Vandeveld Postgraduate Scholarship 2016/17, and Harris and Lauretta and Raymond Earl Parr Memorial Scholarship in Agriculture 2015/2016.

My deep appreciation and love is to my wife, Eyerusalem T. Mitiku, my son Eyu E. Geta, and my little daughter Amen E. Geta for their love, support, patience and encouragement. I am also indebted to my brother Dr. Fikadu G. Tafesse and sister-in-law Elizabeth Tafesse, for their encouragement and assistance in editing my thesis. Furthermore, I thank my parents, mother-in-law, and siblings for their unconditional love, inspiration and encouragement. I extend my appreciation to all my teachers, friends and fellow students here in the University of Saskatchewan for their various support and friendship.

Above all, I praise God, the Almighty, and my Lord Jesus Christ, for his grace and blessings in every respect of my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ARI	Anthocyanin reflectance index
CDC	Crop Development Center
CRI	Carotenoid reflectance index
CT	Canopy temperature
ET	Evapotranspiration
FAOSTAT	Food and Agriculture Statistics
GNDVI	Green normalized vegetation index
GWAS	Genome wide association study
HTC	Heat tolerance index
LSD	Least significant difference
LT	Leaf temperature
NDVI	Normalized difference vegetation index
PRI	Photochemical reflectance index
QTL	Quantitative trait loci
ROS	Reactive oxygen species
SL	Semileafless
SPAD	Soil Plant Analysis Development
TVG	Triangular vegetation greenness
VI	Vegetation index
VIs	Vegetation indices
VPD	Vapor pressure deficit
WBI	Water band index

CHAPTER 1. INTRODUCTION

1.1 Background and motivation

Crop production has been suffering from the consequences of global warming such as heat and drought stresses (Hall, 2004; Mittler, 2006; Wahid et al., 2007). A global scale yield analysis on major cereal crops including, wheat, maize and barley, showed an estimated loss of $5 billion per year due to heat stress from 1981 to 2002 (Lobell and Field, 2007). Several models and climate predictions reveal heat stress due to high air temperature will become more severe in the future than what has been seen and negatively impact crop yield (Tebaldi et al., 2006; Battisti and Naylor, 2009; Lesk et al., 2016). In this global warming scenario, heat resistance is a desirable trait for yield stability particularly for the heat sensitive crops such as pea and other cool season crops.

In pea, heat stress causes impaired photosynthesis, shortened life cycles, abortion of flowers, ovules and pods, and therefore yield loss (Gulilioni et al., 1997; Porter 2005; Bueckert et al., 2015; Jiang et al., 2015; Huang et al., 2017). Interestingly, a preliminary study conducted in Arizona under very hot (~ 42 °C maximum temperature) conditions on 94 members of a pea mapping panel revealed the survival and pod set of 20% of the cultivars which suggested the possibility of identifying heat tolerance traits in pea (Bueckert 2014, unpublished). However, pea heat resistance strategies are mostly unclear, and the identification of traits controlling any adaptive response of cultivars to heat stress is an essential first step for effective breeding and selection of heat robust cultivars.

Heat stress is usually confounded with drought, and evidence is lacking on the combined impact of heat and drought on pea gas exchange, growth, and yield response. Understanding plants' response to individual and combined environmental stresses would lead to improving crops for multiple stresses. To maintain or improve yield performance in drier and hotter climates, new cultivars need to resist individual and combined heat and drought. While pea's sensitivity to heat stress has been documented since the 1950s (Lambert and Lick 1958; Karr et al., 1959), the threshold temperature for yield loss is inconsistent among reports indicating other factors such as soil moisture, and development stage might contribute to heat response.

Pea germplasm has genetically diverse leaf and canopy traits regarding leaf type, canopy habit, determinacy, canopy color, flower color and other morpho-anatomical characteristics (Snoad 1974; Heath and Hebblethwaite 1985; Goldman et al., 1992). These traits may be involved

in heat response. For example, pigments and wax have been reported to have roles in crop adaptation to environmental stresses (Havaux, 1989; Shepherd and Griffiths 2006). Leaf spectral properties and heat response by exogenous wax application, wax removal and pigment degradation may help to determine role of pigments and wax as traits of heat avoidance. Generally, screening and selecting pea germplasm for heat stress resistance requires identification of screening methods and traits. In the past, most heat stress studies focused on reproductive stages and parts (Guilioni et al., 1997; Hall 2004; Jiang et al., 2015), but less effort has been devoted to identifying any potential traits from vegetative organs and canopies.

1.2 Objectives and scope

The overall goal of this thesis was to identify leaf, canopy and biochemical traits involved in pea heat resistance, and determine cultivar response to individual or combined heat and drought stresses. The hypotheses of the study were 1) canopy architecture and leaf type, specifically the semileafless leaf coupled with an upright growth habit contribute to canopy cooling and heat avoidance, 2) wax and pigments enhance pea heat avoidance by improving heat and radiation reflection and minimizing residual transpiration, 3) pea has different threshold temperatures for the various development stages and growth processes, and sufficient water supply minimizes the heat stress effects. The specific objectives of this thesis were:

- To test whether canopy architecture traits specifically leaf type, plant habit, canopy color, and flower color contribute to heat avoidance by maintaining a lower canopy temperature and determine the heat sensitivity level of 24 pea cultivars possessing a range of morphological characteristics,
- To determine the role of leaf pigments including chlorophyll a and b, carotenoid, anthocyanin and wax on pea heat response and yield stability,
- To examine leaf spectral properties and heat response in pea under added wax, shade, and wax removal treatments,
- To determine threshold temperature for the various plant growth and development processes, and assess the effects of development stage on leaf temperature, stomatal conductance, and pigment and wax formation,
- To examine individual or combined effects of heat and drought stresses on gas exchange, growth and yield of selected pea cultivars.

To test the above hypothesis and attain the specific objectives, five activities (experiments) were conducted during 2014-2017 under field and growth chamber conditions: 1) Testing twenty-four pea cultivars in the field across six environments to search for morphological and biochemical traits involved in canopy cooling and heat avoidance (Chapters 3 and 4), 2). Exogenous wax application, dewax, and shade effects on leaf spectral properties and heat avoidance in pea under field condition (Chapter 5), 3) Individual and concurrent effects of heat and drought on gas exchange, growth and seed yield of field pea under controlled environment (Chapter 6), 4) Determination of threshold temperature for various development stage and growth processes (Chapter 7).

CHAPTER 2. LITERATURE REVIEW

2.1 Origin, production, and agronomic characteristics of pea

Field pea (*Pisum sativum* L., 2n = 14) is a cool season annual legume in the family of Leguminosae, the third largest angiosperm family with over 800 genera and 18,000 species (Gritton, 1980; Lewis et al., 2005). Pea is one of the oldest domesticated crops and has been cultivated since the Neolithic period, as early as 7000 to 6000 BC (Zohary and Hopf, 1973; Gritton, 1980; Baldev 1988; Smýkal et al., 2012). Although the exact center of origin is largely unknown, pea is believed to have originated somewhere in the Mediterranean basin and Near East (Helback, 1959; Zohary and Hopf, 1973; McPhee, 2003). In these regions, among other food legumes and cereals, pea had been part of the daily dietary components of the early community (Zohary and Hopf, 2002). Pea cultivation was expanded from the Fertile Crescent to Russia and westwards into Europe, to ancient Greece and Italy from where pea was further spread to northern and western Europe, and to Persia, India and China (Zohary and Hopf, 2002). Although it grows in many parts of the world, pea is a cool season crop, and mainly adapted in semi-arid temperate conditions, in the moist dark brown and black soil zones (McPhee, 2003).

Currently, the major pea producing countries are Canada, Russian Federation, China, India, and the United States of America (FAOSTAT, 2016). Pea production in Canada was started about 100 years ago on limited acreage (Goodwin, 2008), and the production began to increase after World War II. From 1990 to 2016, Canadian pea production was increased over 17-fold, from 0.27 to 4.6 million tonnes, and currently Canada accounts for a third of global dry pea production (FAOSTAT, 2016), and nearly all of such production comes from western Canada (Goodwin, 2008).

Pea germplasm has genetically diverse morphological characteristics in terms of growth habit, canopy architecture, leaf type, canopy and colors, and seed market classes (Snoad 1974; Heath and Hebblethwaite 1985; Goldman et al., 1992; Warkentin et al., 2015). Generally, four simply inherited characters determine the pea market classes: the presence or absence of pod parchment, flower color, leaflets occurrence, and the type of starch in the seed (Warkentin et al., 2015). Commercial pea cultivars have either normal or semileafless leaf types. The normal leaf consists of stipules, petiole, leaflets, and tendrils, whereas in semileafless cultivars leaflets are replaced by tendrils, and the plant uses the stipule, a basal leaf surface, for photosynthesis (Goldman et al., 1992; McPhee et al., 2003; Cote et al., 1992; Gourlay et al., 2000; Heath and

Hebblethwaite, 1985). In the early 1980s, the first semileafless pea cultivar was released for commercial use in England (Martin et al., 1994).

Today, most commercial pea cultivars in North America, Europe, and Australia have the *afila* gene for the semileafless trait (Snoad 1974). Semileafless pea is preferred for lodging resistance, and its unique canopy structure permits more light penetration and canopy aeration (Cote et al., 1992). Also, the semileafless trait may help to conserve water by minimizing evapotranspiration (Wilson et al., 1981) due to increased tendrils and reduced leaf blades (Heath and Hebblethwaite, 1985). Semileaflessness was also positively correlated with disease resistance (Snoad, 1974).

Regarding growth habit, pea can be determinate or indeterminate (Zohary and Hopf 2002), and the majority of pea cultivars are indeterminate (Cousin 1997). Most of the indeterminate spring habit cultivars in USA and Canada require longer seasons, 90 to 100 days to maturity while determinate cultivars have shorter life cycles of 80 to 90 days (McPhee et al., 2003). Indeterminate cultivars have a vining nature and their vine length is longer than determinate cultivars (McPhee et al., 2003). Regarding flower color, most pea cultivars have either white or reddish to purple flower colors (Zohary and Hopf 2002). For canopy habit, pea can broadly be categorized either as upright or vining (Davies 1977), and the upright nature due to semileafless, which facilitates enhanced aeration within the canopy and may help lower canopy temperature. For canopy color, pea has a range of canopy color from dark-green to yellowish, and canopy color is associated with green pigment concentration (Sanchez et al., 2001). Pigments may contribute to the reflection of radiation and heat load, or to protection of plant photosystems (Demmig-Adams and Adams 1996).

2.2. Nutritional value and uses of pea

Pea, along with other pulse crops, has long been an important component of the human diet, primarily for its nutritional value as a source of starch, protein and other essential nutrients in the edible seed (Gritton, 1980; Cousin, 1997; McPhee et al., 2003; Dahl et al., 2012). Pea seed is rich in thiamin and amino acids such as lysine and tryptophan, lacking in most cereals (McPhee et al., 2003). Pea seed is rich in protein, and the protein concentration ranges from 13.7 to 30.7% of seed dry matter (Tzitzikas et al., 2006). Pea seed is rich in slow digestible starch (50%), soluble sugars (5%), and fiber content (20%), and is low fat (2%) (McPhee et al., 2003; Bastianelli et al., 1998; Smýkal et al., 2012). Pea seed has oil content ranging from 1.5% to 3.7%, which suggests current pea cultivars are not suitable to be used as an oilseed crop (Welch and Griffiths, 1984;

Yoshida et al., 2007). Generally, pea has a great nutritional value both as human food and animal feed.

Like most legume crops, in addition to its nutritional benefit, pea has long been grown as a rotation crop with cereals to break cereal disease and weed cycles (McPhee et al., 2003). Pea improves soil fertility mainly by forming a symbiotic relationship with the bacterium, *Rhizobium leguminosarum*, which has the ability to fix atmospheric nitrogen into plant-available forms and eliminate the requirement for synthetic nitrogen fertilizer application (Goodwin, 2008)

2.3 Climate change and crop heat stress

One of the effects of global warming is climate change which has led to aggravated drought and heat stresses in many regions of the world (Battisti and Naylor, 2009). Several models and climate predictions reveal the intensity, frequency and duration of heat waves and extreme temperature events will become more common in the future compared to recent years, and negatively impact crop yield (Tebaldi et al., 2006; Battisti and Naylor, 2009; Lesk et al., 2016). Generally, the average maximum air temperature will continue to rise at a rate of 0.3 ℃per decade for the next 100 years (Lobell and Lobell, 2012).

Crop production is fundamentally sensitive to climate change, and it mainly suffers from heat and drought stresses (Hall, 2004; Mittler, 2006; Wahid et al., 2007). A global scale yield analysis on major cereal crops, wheat, maize and barley, estimated a loss of $5 billion per year due to warming from 1981 to 2002 (Lobell and Field, 2007), and extreme heat occurrences from 1964 to 2007 reduced global cereal production by 9% (Lesk et al., 2016). High temperature stress usually occurs in combination with high irradiance and drought; all exacerbate the level of damage to crop growth and development (Hall, 1992; Mittler, 2006; Prasad et al., 2008). For instance, in water deficiency, heat stress is aggravated by the inability of the plant to effectively reduce canopy temperature by plant transpiration (Kashiwagi et al., 2008).

2.4. Effect of heat stress on pea production

Temperature affects the rate of plant growth and development. Warmer temperatures shorten the plant life cycle and will mostly result in a reduced yield (Wahid et al., 2007; Bueckert et al., 2015; Huang et al., 2017). As a cool season crop, pea is sensitive to heat stress, and it starts to suffer the stress whenever the daily maximum air temperature exceeds a certain maximum threshold, which is usually lower than the maximum threshold temperature of other cool season

legumes including faba bean, lentil and chickpea (Pumphrey and Raming, 1990; Guilioni et al, 2003; Sadras et al., 2013; Bueckert et al., 2015; Sita et al., 2017). A transitory or extended elevation of maximum air temperature brings a series of altered morpho-anatomical, physiological, biochemical and molecular responses (Guilioni et al., 1997; Wahid et al., 2007; Hasanuzzaman et al., 2013).

Heat stress affects pea's growth and development at many developmental stages (Guilioni et al., 2003). Primary effects of heat stress are impaired photosynthesis, shortened flowering duration and accelerated senescence, flower and pod abortion, and consequently a remarkable yield loss of up to 70% (Nonneke et al., 1971; Pumphrey and Ramig, 1990; Guilioni et al., 2003; Sadras et al., 2013; Bueckert et al., 2015). Heat stress during reproduction reduces pollination, and causes abscission of floral buds, flowers and pods, all of which result in substantial yield loss (Guilioni et al., 1997; Nakano et al., 1998). Although heat stress affects crop performance at any stage from germination to maturity, the reproductive phase is more affected by stress compared to the vegetative phase (Guilioni et al., 2003; Wahid et al., 2007).

There are a number of target sites for high temperature-induced damage in photosynthesis such as disturbance in the CO_2 fixation system, photophosphorylation and the electron transport chain (Nash et al., 1985; Feller et al., 1998). At moderately high temperatures, injury or death may occur only after long-term exposure but at more extreme temperatures, severe cellular injury and even cell death may occur within a very short time (Wahid et al., 2007). Direct injury due to high temperature includes protein denaturation and aggregation, and increased fluidity of membrane lipids (Hall, 2011). Indirect or slower heat injuries include inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein degradation and loss of membrane integrity. Heat stress also causes plant stunting, reduced ion flux, inhibition of PSII, production of toxic compounds and reactive oxygen species (ROS) (Wahid et al., 2007).

2.5 Heat stress threshold temperature of pea

There are three cardinal temperatures that influence plant growth and development: 1) the base temperature (also called the lower temperature threshold), 2) the optimum temperature, and 3) the upper threshold temperature (Porter and Gawith, 1999). These temperatures vary among plant species, and each plant species has an optimum range of temperature specific to the plant's growth stage (Wahid et al., 2007). Generally, the optimum temperature for cool season crops ranges from 10-30 °C, and in most cases a daily maximum temperature of 25-28 °C is considered

as a threshold level for heat stress (Sadras et al., 2013; Hasanuzzaman et al., 2013; Bueckert et al., 2015). Cool season legumes include chick pea, faba bean, lentil, and faba bean (Wang et al., 2006; Fang et al., 2010; Bhandari et al., 2016). The upper threshold temperature refers to the temperature beyond which seed germination, seedling and vegetative growth, flowering, fruit set, and fruit ripening are critically impaired (Wahid et al., 2001).

Although pea heat stress sensitivity has been extensively studied and reported since the 1950s, reports were inconsistent on the threshold temperatures for yield reduction. Lambert and Lick indicated a temperature range of 27-32 ºC led to significant yield loss, but they also emphasized the exposure duration, as it was equally important as the temperature intensity, and the longer the duration of exposure, the greater was the yield loss. Nonnecke et al (1971) indicated continued growth at 27/17 day/night temperature under controlled environment resulted in a 50-70% yield reduction. Stanfield et al (1966) reported pea yield reduction started whenever the air temperature reached 16 ºC or beyond, which is a relatively low threshold temperature. Ridge and Pye (1985) indicated a 1 ºC increase in mean air temperature during flowering stage resulted in a 0.6 tonnes hectare^{-1} yield reduction. Pumphery and Raming (1990) suggested 25.6 °C as a maximum threshold temperature beyond which yield started to decline, and the decline became exponential beyond 27ºC. On the other hand, Jeuffroy et al (1990) indicated, under a controlled environment, yield reduction was evident only after the air temperature reached 31 °C or more. Recently, Sadras et al (2012) indicated mean air temperature for grain yield reduction during the reproductive phases was 25 °C, and Bueckert et al (2015) suggested 28 ºC as a threshold temperature for yield reduction under dryland condition, which is high relative to earlier studies. In a controlled environment study, Jiang et al (2015) indicated the critical temperature for a significant reduction in pollen germination and pollen tube length was 36 °C.

2.6. Plant adaptation to heat stress

To cope with environmental stress, plants have developed various phenological, physiological and biochemical modifications to survive and perform under marginal environments (Hasanuzzaman et al., 2013). There are three different categories of heat adaption: heat escape, heat avoidance, and heat tolerance (Shepherd and Griffith, 2006; Bueckert and Clark, 2013). Heat escape is a phenological mechanism that involves early flowering and maturity before the onset of severe heat. However, early maturity usually associates with yield trade-off especially in favourable environments, but is desirable in situations where heat stress occurs at sensitive stages

such as anthesis, flowering and pod setting (Bueckert and Clark, 2013). Crop management practices such as shifting and re-adjusting seeding date, planting density, soil and irrigation management are effective in facilitating escape from heat stress (Wahid et al., 2007; Sadras and Dreccer, 2015). The breeding strategies of selection for short flowering duration and early maturity enhance plants' escape from stress.

Heat resistance mechanisms can be categorized as avoidance and tolerance strategies (Bueckert and Clark, 2013). The avoidance mechanism involves modification of internal conditions that keeps the plant cells unstressed regardless of the presence of the external stress, for example avoiding heat through enhanced transpiration cooling during high temperature condition. The other plant heat avoidance strategy is heat reflectance, which was reportedly associated with leaf surface wax which effectively reflects both UV and near infrared radiation, reducing leaf temperature (Holmes and Keiller, 2002; Sanchez et al., 2001; Shepherd and Griffith; Gamon and Surfus, 1999). Heat tolerance enables biological functionality, and survival, but results in loss in agronomic productivity of the crop while under the heat stress. More detail on the different mechanisms of plant adaptation to heat stress is presented in table 2.1.

Table 2.1. Crop heat adaptation strategies, heat escape and resistance. The heat resistance is either by avoidance or by tolerance (Bueckert and Clark, 2013; Wahid et al., 2007).

Heat escape	**Heat resistance**		
Shortening life cycles	Avoidance		Tolerance
• Early flowering	a	Maintaining temperature balance	• Yield penalty
• Early maturing		• Transpiration cooling	• Dormancy
• Short flowering duration		• Radiation reflectance and minimized absorbance by epicuticular wax	
		• Conserve water and minimized canopy temperature	
	b	Compensating yield by maintain growth and development for a longer duration	
		• Long flowering duration	
		• Indeterminacy	

2.7. Heat acclimation

Under unfavorable conditions such as drought and heat stresses, plants undertake modification of physiological and biochemical processes which gradually improves their ability to tolerate the stress, referred to as acclimation (Hasanuzzaman et al., 2013). Heat acclimation generally occurs through gradual exposure to a non-lethal treatment (Chen et al., 1982). According to Sangwan et al (2002), the heat acclimation process initiates by the perception of the high temperature signals and transduction of the signals into biochemical processes that finally lead to the development of heat tolerance. Among other processes, heat acclimation involves a significant readjustment of thylakoid membranes, and adaptive changes of lipid composition (Larkindale and Huang, 2004). During the process of heat acclimation, the threshold temperature for the maintenance of membrane fluidity and other biochemical processes rises (Raison et al., 1982). Generally, the mechanisms involved, and effects of heat acclimation processes are not sufficiently studied.

2.8. Heat stress indicators and associated traits

2.8.1. Canopy temperature

Canopy temperature (CT) is basically a diagnostic trait to indicate the relative adaptability of a genotype to the environment (Jackson et al., 1981). Under hot environments, a cooler canopy was associated with higher yield (Bahar et al., 2008). Infrared thermometry to quantify canopy temperature differences among wheat cultivars has been used for yield prediction under hot, irrigated environments (Reynolds et al., 1994). Canopy temperature measurement is rapid, simple and inexpensive (Amani et al., 1996; Reynolds et al., 2001), and considered as an ideal method to indicate crop stress level under field conditions. The primary limitation in CT use is that the method is sensitive to environmental factors such as soil water availability, air temperature, relative humidity, and radiation incidence, requiring relatively cloud-free, windless days to obtain reliable data (Reynolds et al., 2001).

The CT measurement result is usually presented as canopy temperature depression (CTD) in association with the ambient air temperature. Canopy temperature depression, the difference between the canopy temperature (Tc) and the ambient air temperature (Ta), is an indicator of overall plant physiological status. The canopy temperature depression is negative when the canopy is cooler than the air indicating no stress, and positive when the canopy is hotter than the air

temperature indicating a heat stressed canopy (Jackson et al., 1981; Idso 1982; Howell et.al., 1986; Amani et al., 1996; Balota et al., 2007).

2.8.2 Stomatal conductance

Stomatal movement affects rates of inflow of CO_2 into the stomatal cavity and to the site of carboxylation, and outflow of water through the leaves in the process known as transpiration (Amani et al., 1996). Stomatal conductance is a key variable that determines the rate of net photosynthesis and thus the global carbon cycle and plant carbon metabolism. Regarding heat stress, stomatal conductance has a direct effect on transpirational cooling; a strong relationship exists between stomatal conductance and canopy temperature (Amani et al., 1996). The closure of stomata may increase leaf temperature depending on the radiation load on the canopy but will result in a better water economy or increased transpiration efficiency (Yoo et al., 2009). Stomatal conductance can be monitored instantaneously over a growth period using several tools such as a hand-held porometer, a flux tower, and infrared gas analyzer.

2.8.3. Leaf pigments

The composition and concentration of photosynthetic pigments can be used to provide valuable insight to determine the physiological status of a plant (Sims and Gamon, 2003; Osório et al., 2012). According to Sanchez et al., (2001), leaf color has been reported as a trait that improves crop productivity under drought stress. In barley, higher grain yield under conditions of heat stress is associated with lighter green leaves, while higher yield in non-stressed conditions is associated with dark green color (Ceccarelli, 1987). Healthy stay-green plants are more productive in terms of yield and biomass accumulation (Thomas and Smart, 1993) and this feature delays senescence which plays a key role in determining the final yield of the plant. Stay green cultivars are well adapted to drought and heat stressed conditions (Kumar et al., 2010).). In barley, leaf color has been used as a character to identify drought-resistant genotypes (Ceccarelli, 1987). Pale green leaves in barley have been associated with a lower concentration of chlorophyll and higher chlorophyll *a*/*b* ratios (Watanabe et al., 1995). In genotype evaluation, variation in pigment content may provide information concerning the physiological state of leaves especially under stress conditions (Penuelas, et al., 1993). Chlorophyll degrades faster than carotenoids when plants are under stress or during leaf senescence (Chalker-Scott, 1999).

Chlorophyll plays a key roles in the light harvesting and energy transduction processes of photosynthesis (Sims and Gamon, 2003). Generally, leaf chlorophyll concentration is a direct measure of pigment abundance and composition in leaves. It is associated with photosynthetic activity, relative degree of senescence, and yield potential. Non-destructive and simple chlorophyll meters like SPAD (soil and plant analyzer development) allow field-based chlorophyll content assessment without damaging plant tissues. The estimates from these devices are reported to be accurate and strongly correlate with laboratory based destructive methods. Chlorophyll is a heat sensitive trait, and heat stress generally leads to pigment reduction (Karim et al., 1999; Dutta et al., 2009). Such a loss in chlorophyll concentration arose either due to decreased biosynthesis or due to pigment degradation, or a combination of the above scenarios due to the heat stress (Karim et al., 1999). Chlorophyll degradation generally results in impaired photosynthesis and oxidative damage, and thus to reduced growth and yield (Berry and Bjorkman, 1980; Guo et al., 2006; Wahid et al., 2007).

Carotenoids are among the abundant naturally existing pigments, with over 700 members, and they are mainly C40 lipophilic isoprenoids and are synthesized in all photosynthetic organisms (Khoo et al., 2011). Generally, carotenoids have two primary roles in photosynthetic organisms. First, they function as accessory light-harvesting pigments, effectively extending the range of light absorbed by the photosynthetic apparatus. Secondly, they play a photoprotective role by scavenging harmful oxygen species formed within the cells owing to environmental stress (Cazzonelli and Pogson, 2010; Havaux, 2014). The use of solar energy in photosynthesis depends on the ability to safely dissipate excess energy as heat, and the key dissipation process in the natural environment is mediated by a particular group of carotenoids, the xanthophylls (Powles, 1984; Deming-Adam and Adams, 1996; Misra et al., 2006). Carotenoid-deficient photosynthetic organisms are highly photosensitive, suffering extensive photo-damage (Havaux, 1989). For example, a carotenoid molecule called zeaxanthin, formed in response to environmental stress, was identified for its involvement in the dissipation of damaging excess energy under stress conditions (Deming-Adam and Adams, 1996).

Anthocyanins are secondary metabolites and are part of the large phenolic family, flavonoids. Generally, anthocyanin biosynthesis associates with various environmental signals (Mol et al., 1998; Weiss, 2000). Drought stress, radiation stress, cold stress, heat stress, wounding (Creelman et al., 1992; Ferreres et al., 1997), and pathogen infection (Dixon et al., 1994) have all

been reported to induce anthocyanin production (Chalker-Scott, 1999; Hasegawa et al., 2001). On the other hand, stressful environments also trigger the formation of harmful reactive oxygen species (ROS), and free radicals (Tripathy and Oelmuller, 2012). To protect plants from the harmful effects of ROS, a high level of anti-oxidants is needed, and anthocyanins are reported to play an anti-oxidant role through scavenging reactive oxygen species (Yamasaki et al., 1996; Yamasaki, 1997). Anthocyanins also protect sensitive plant tissues by screening damaging UV radiation (Singh et al., 1999), and their concentration increases in response to high temperature stress (Hosseinian et al., 2008). Anthocyanin concentration tends to be high in young leaves that also have low photosynthetic rates (Gamon and Surfus, 1999; Chalker-Scott, 1999).

2.8.4. Wax

Cuticular waxes form the outermost barrier over plant surfaces and are among the most important traits for plant survival under stressful environments such as drought and heat (Jenks et al., 2000; Buschhaus and Jetter, 2011). Plant cuticular waxes are composed of very long chain aliphatic lipids with a length of C20-C34 (Gonzalez et al., 1996). Generally, plant cuticular wax has two distinct physical layers called intracuticular and epicuticular wax; the latter is deposited on the outer surface (Buschhaus and Jetter, 2011). Wax composition varies between crop species, cultivars, organs, and developmental stages, which all balance non-stomatal water loss with various other physiological processes (Ebercon et al., 1977; Gonzalez et al., 19 96; Gniwotta et al., 2005; Buschhaus and Jetter, 2011). Waxes are mixtures of very long-chain compounds specific to plant species and even cultivars. The waxes on the upper and lower leaf surfaces can have different composition, such that the adaxial surface has more alcohols and the abaxial surface has more alkanes (Gniwotta et al., 2005). In pea, waxes are composed of alkanes (50%), esters and aldehydes (18%), primary alcohols (19%), secondary alcohols (7%) and free acids (6%; Macey and Barber, 1970).

Although genetic variation contributes to tremendous variation in wax components in a range of crops, wax development also depends greatly on environmental factors (Shepherd and Griffiths, 2006). The two most commonly reported roles of wax were protection against excess radiation through the reflection of visible and infrared wavelengths (Jefferson et al., 1989; Shepherd and Griffiths, 2006), and minimizing water loss through reduced residual transpiration (Sanchez et al., 2001; Hasanuzzaman et al., 2017). Generally high wax concentration associated

with decreased canopy temperature (Sanchez et al., 2001). Blueish, or whitish leaf color has been used to visually identify glaucousness or waxy leaf surface in several crops (Jenks et al., 2002; Buschhaus and Jetter, 2011; Willick et al., 2017). Sanchez et al (2001) indicated epicuticular wax as a mechanism to decrease residual transpiration water loss in pea. Generally, glaucousness (or waxy leaves) helps to maintain high water potential and can therefore be considered as a drought tolerance trait (Richards et al., 1986; Ludlow and Muchow, 1990), and indirectly as a heat tolerance trait. Richards et al (1986) indicated a 0.7 °C difference in leaf temperature between waxy and non-waxy wheat cultivars.

2.8.5. Spectral reflectance and leaf optical properties

Spectral reflectance from a plant's leaf or canopy at different wavelengths indicates plant health, vigor, and overall physiological status (Filella et al., 1995; Kokaly and Clark, 1999; Osório et al., 2012). The spectral reflectance at different wavelength can be used to characterize overall crop status and the functional regions of the electromagnetic spectrum (Babar et al., 2006). Pigments in leaves absorb light strongly in the photosynthetically active radiation (PAR) but not in the NIR (750 nm–2500 nm) region (Knipling, 1970) which results in a higher reflection of radiation in the near infrared (NIR) compared to the visible region of the spectrum (Carter, 1998). Plant reflectance is governed by leaf surface properties and internal structure, as well as by the concentration and distribution of biochemical components (Jacquemoud and Ustin 2001; Holmes and Keiller 2002), and thus remote sensing of reflected light can be used to assess both the biomass and the physiological status of a plant (Filella, et al., 1995). Reflectance from plant canopies and individual leaves varies depending on the level of environmental stress and plant physiological status (Sim and Gamon, 2003). Several reports show associations between spectral indices and yield of genotypes in a range of moisture-stressed and non-stressed environments (Royo et al., 2003; Babar et al., 2006).

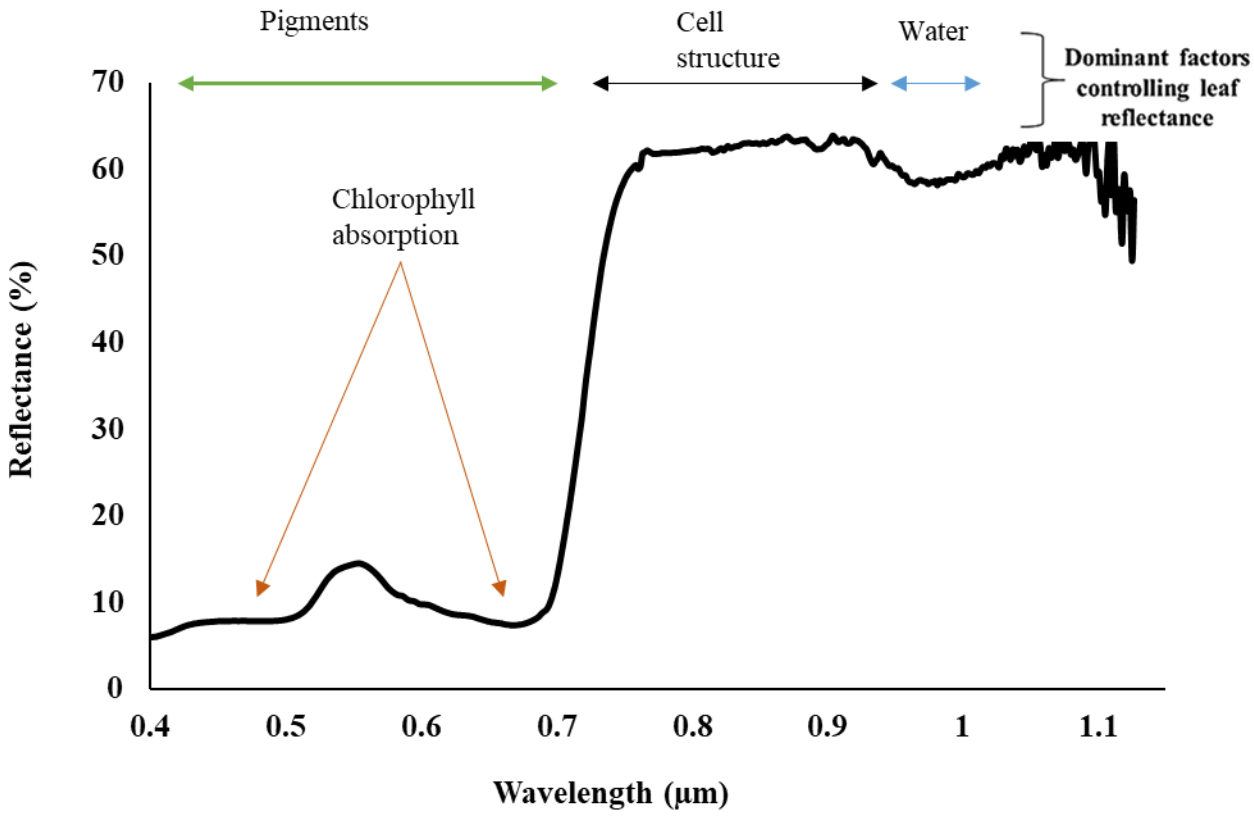


Figure 2.1. Typical spectral reflectance of a healthy and green leaf in the visible and near infrared regions of light spectrum.

Spectral reflectance measurements provide information that may be essential to estimate biomass, the amount of absorbed radiation, and the plant's photosynthetic efficiency (Wiegand et al., 1990; Wiegand et al., 1991; Price and Bausch, 1995; Reynolds et al., 2001). Some of the physiological parameters that can be inferred from spectral reflectance include nutritional status of the plant and level of environmental stress through estimations of chlorophyll and carotenoid concentration (Chappelle et al., 1992; Reynolds et al., 2001; Babar et al., 2006). Grain yield has also been estimated using spectral reflectance indices during different growth stages of the crop (Osborne et al., 2002; Babar et al., 2006). Non-stressed vegetation absorbs most of the visible light, while reflecting most of the NIR light. Conversely, stressed vegetation absorbs only a small portion of the visible and a larger proportion of NIR light (Reynolds et al., 2001). Spectral reflectance data can be used to compute a variety of vegetative indices that are well-correlated with agronomic and biophysical plant parameters (Wiegand et al., 1990; Wiegand et al., 1991; Price and Bausch, 1995).

Vegetative indices (VIs) calculated from spectral reflectance indicate the overall physiological status and the plants stress tolerance level (Gamon et al., 1997; Xue and Su 2017).

Generally, three main categories of traits can be estimated from the different VIs based on the reflectance wavelengths (Xue and Su 2017). The first group of VIs includes those indices derived from the visible spectral region, including photochemical reflectance index (PRI), normalized pigment and vegetation index, and carotenoid reflectance index (Gamon et al., 1997; Peñuelas et al., 2004). The second group involves reflectance in the visible and near infrared regions from which Vis are derived that indicate vegetation vigor, greenness, and rate of senescence (Babar et al., 2006). The most common VIs of this group includes NDVI and its derivatives. The third group involves VIs derived from the near infrared region reflectance, which are proxies mainly for the tissue water status (Penuelas et al., 1997; Zarate-Valdez et al., 2012). The typical index in this group is water band index (WBI) (Penuelas et al., 1997).

2.9. Strategies to improve plant heat tolerance

Cultural practices such as seeding time, plant population density, soil and irrigation management, can minimize stress effects. However, sustainable improvement of heat tolerance can only be attained by combining cultural practices with genetic improvement. Generally plant responses to heat stress are well understood, but the progress of developing heat-resistant cultivars lags behind. On the positive side, pea has a remarkable genetic variation in physiological, morphological and biochemical characteristics that can be deployed in new cultivars to confer heat resistance. Traditionally, most plant breeding programs have focused on the development of cultivars with high yield potential under optimal conditions (Warren, 1998). However, global warming and climate trends necessitate the improvement of plants for marginal environments (Blum, 1988). Breeding success for stress resistance largely depends on understanding of the physiological mechanisms and genetic bases of stress tolerance at the whole-plant, cellular and molecular levels. Currently there is only limited information on the genetic basis of heat tolerance.

2.9.1. Conventional breeding

Most abiotic stress tolerance traits are complex, controlled by more than one gene, and highly influenced by environment, and so the quantification of tolerance is difficult (Barnabas et al., 2008; Fleury et al., 2010). Direct selection under field condition may not be feasible due to the huge impact of the environment. For example, no consistent high-temperature conditions can be maintained under field conditions. Moreover, stress tolerance is usually phenology dependent, and heat sensitivity is different at the different development stages. A common approach is to make

selection under the heat stressed environment throughout the crop development stages (Ehlers and Hall, 1998). The other approach is selection under a controlled environment (growth chamber), by maintaining the required level of temperature throughout the selection process. In either case, selection for heat tolerance requires a reliable selection method and selection criteria such as the use of a heat tolerance index (HTI, Fernandez, 1992). Breeding to improve for heat resistance may lead to the development of a heat tolerant cultivar. Selection for flowering (i.e. flower number) and fructification (number of well-formed pods) can be efficient in developing tolerant varieties for heat-sensitive species (Hall, 1993).

2.9.2. Physiological breeding based on favourable traits

Despite the understanding and identification of genes of major effect such as for disease resistance, the genetic basis of heat tolerance is poorly understood. Physiological traits associated with heat resistance may be used for improving crop heat resistance. Traits represent a favorable combination of alleles for a given plant character such as a cooler canopy temperature (Cossani et al., 2012). While the outcome of combining heat-adaptive physiological traits may sometimes be difficult to predict, crosses made between parents with variable but complementary traits would enhance the chance of a cumulative gene action (Reynolds et al., 2009). Thus, the physiological breeding approach aims to combine several associated traits that may contribute to overall heat tolerance; and it requires that each trait reasonably contributes to heat tolerance. Such traits include epicuticular wax involved in heat dissipation, pigments involved in protection of photosystems from heat damage.

CHAPTER 3. HEAT STRESS TOLERANCE OF FIELD PEA DEPENDS ON CANOPY ARCHITECTURE AND LEAF TYPE

3.1 Introduction

Crop production suffers from heat stress (Hall 2004; Wahid et al., 2007). A global scale yield analysis on the major cereal crops, wheat, maize and barley, demonstrated an estimated loss of $5 billion per year due to warming from 1981 to 2002 (Lobell and Field 2007), and extreme heat occurrences from 1964 to 2007 resulted in a 9% reduction of global cereal production (Lesk et al., 2016). As a cool season crop, pea is particularly sensitive to heat stress, and it starts to suffer the stress whenever the daily maximum air temperature exceeds a threshold, usually 25 to 28 ºC in the field (Pumphrey and Ramig 1990; Guilioni et al., 2003; Sadras et al., 2013; Bueckert et al., 2015). A transitory or extended elevation of temperature beyond a defined threshold brings a series of altered morphological, physiological, biochemical and molecular responses, ultimately leading to retarded plant growth and then loss of economic yield (Guilioni et al., 1997; Wahid et al., 2007; Hasanuzzaman et al., 2013).

While pea's sensitivity to heat stress has been documented since the 1950s (Lambert and Linck 1958; Karr et al., 1959), screening and selecting pea germplasm for heat stress resistance requires identification of screening methods and traits. In the past, most heat stress studies focused on reproductive stages and parts (Guilioni et al., 1997; Hall 2004; Jiang et al., 2015), but less effort has been devoted to identifying potential traits from vegetative organs and canopy parameters. Identification of traits controlling any adaptive response of cultivars to heat stress is an important first step for effective breeding and selection for desirable traits.

Pea germplasm has diverse leaf and canopy architecture in terms of canopy habit, leaf type, and determinacy (Snoad 1974; Heath and Hebblethwaite 1985; Goldman et al., 1992). Most commercial pea cultivars in North America, Europe, and Australia have the *afila* gene for the semileafless trait (Snoad 1974), where leaflets are reduced to tendrils and the plant uses the stipule, a basal leaf surface, for photosynthesis (Goldman et al., 1992). The normal (entire) leaf type in pea has two, three or four sets of leaflets (Cote et al., 1992; Gourlay et al., 2000). The semileafless trait may help to conserve water by minimizing evapotranspiration (Wilson et al., 1981) due to increased tendrils and reduced leaf blade from stipules only. For canopy habit, pea can broadly be categorized either as upright or vining (Davies 1977), and the upright nature may facilitate enhanced aeration within the canopy and help lower canopy temperature.

A thicker stem may help to conduct and keep water in the stem to maintain the plants' canopy temperature within a certain range (Reicosky et al., 1980). Canopy color may be associated with pigments composition and concentration, which may involve in protection of vital components from heat load damage (Powles, 1984; Demmig-Adams and Adams 1996). Such diverse morpho-physiological features of pea can be investigated under various environments to identify potential traits associated with canopy cooling and greater yield under stress. The specific objectives of this study were 1) to test whether canopy architecture traits, specifically leaf type, plant habit, canopy color, and flower color contributed to heat avoidance by maintaining a lower canopy temperature, and 2) to determine the heat sensitivity level of pea cultivars possessing a range of heat avoidance traits. I hypothesized canopy architecture and leaf type, specifically the semileafless leaf coupled with an upright growth habit, contribute to canopy cooling and heat avoidance, and thus reduce growth and yield losses under heat and drought stressed environments.

3.2 Materials and Methods

3.2.1 Plant materials

Twenty four pea cultivars representing the various combinations in commercial pea regarding leaf type, plant growth habit, determinacy, canopy color, flower color and other agronomic characteristics such as flowering date, disease resistance and seed market class were used in this study. Most of the cultivars were adapted and widely grown in many parts of the world including Australia, Europe, USA and Canada. The cultivars name, specific characteristics, and origin are presented in Table 3.1.

3.2.2 Environments and growing conditions

A field study was conducted for three years (2014-2016) at two locations: Rosthern (52º66'N, 106º33'W) and Saskatoon (52º12'N, 106º63'W), Saskatchewan, in western Canada. The field study consisted of six experimental environments: 2014 at Rosthern (R14); 2014 at Saskatoon (S14); 2015 at Rosthern (R15); 2015 at Saskatoon (S15); 2016 at Saskatoon (S16); and 2016 at Saskatoon with normal seeding date (SN16). All environments except SN16 involved a purposely delayed seeding date by three to four weeks from the normal seeding time for pea in Saskatchewan, so that the first flower and flowering duration would be delayed into mid-July to early August, at which time daytime air temperatures were most likely to exceed 25 ℃. The normal

seeding date in the area is mid-April to early-May. The experimental design at each environment was a randomized complete block with four replications. Plot size was 1.37 m width × 3.66 m length. In order to minimize errors associated to edge effects such as shading or wind effects, the experimental plots at each environment were surrounded (bordered) by other pea plots. Further details including location, year, planting date and weather conditions of each environment are presented in Table 3.2.

Table 3.1. Description of leaf type, plant habit, canopy color, flower color, and origin of the 24 pea cultivars used in this study.

Cultivars	**Leaf type**	**Plant habit**	**Canopy color**	**Flower color**	**Origin**
03H107P-04HO2026	Semileafless	Upright	Bright-green	Red	Australia
03H267-04HO2006	Semileafless	Upright	Bright-green	Red	Australia
40-10	Normal	Vining	Dark-green	Red	Western Europe
Aragorn	Semileafless	Upright	Dark-green	White	USA
CDC Golden	Semileafless	Upright	Dark-green	White	CDC, Canada
CDC Meadow	Semileafless	Upright	Dark-green	White	CDC, Canada
CDC Sage	Semileafless	Upright	Dark-green	White	CDC, Canada
CDC Vienna	Semileafless	Upright	Dark-green	Red	CDC, Canada
Delta	Semileafless	Upright	Dark-green	White	Western Europe
Eclipse	Semileafless	Upright	Bright-green	White	Western Europe
KASPA	Semileafless	Upright	Dark-green	Pink	Australia
MFR043	Normal	Upright	Bright-green	White	CDC, Canada
Mini	Normal	Vining	Dark-green	White	USA
MPG87	Normal	Vining	Dark-green	White	AAFC, Canada
Naparnyk	Normal	Vining	Bright-green	White	Eastern Europe
Rally	Normal	Vining	Dark-green	White	USA
Superscout	Normal	Vining	Dark-green	White	USA
TMP 15116	Normal	Vining	Bright-green	White	Eastern Europe
TMP 15179	Semileafless	Upright	Dark-green	White	Eastern Europe
TMP 15181	Normal	Vining	Bright-green	White	Eastern Europe
TMP 15202	Normal	Upright	Bright-green	White	Eastern Europe
TMP 15206	Semileafless	Vining	Dark-green	White	Eastern Europe
TMP 15213	Normal	Upright	Bright-green	Red	Eastern Europe
Torsdag	Normal	Vining	Bright-green	White	Western Europe

CDC stands for Crop Development Center; TMP stands for a gene bank temporary accession designation.

In the fall prior to seeding, plots were treated with herbicides: the recommended rate of granular Edge (ethalfluralin), and one-third of the recommended rate of Pursuit (imazathapyr).

Fertilizer was not applied but each of the pea trial sets were spring seeded into cereal stubble and inoculated with commercial rhizobia for nitrogen fixation. In-season weed control slightly varied among the environments. In 2014: the recommended rate of glyphosate was sprayed one week prior to seeding both at Rosthern and Saskatoon. Four weeks after seeding, the recommended rates of Pursuit and Poast (sethoxydim) were applied at Rosthern, and Viper (imazamox + bentazon) was applied at Saskatoon. In 2015: the recommended rate of glyphosate was applied one week after seeding in both locations, and four weeks after seeding, the recommended rates of Pursuit and Poast were applied in Rosthern, and Viper (imazamox + bentazon) was applied in Saskatoon. In 2016: a full rate of glyphosate was sprayed one week after seeding, and Viper was applied four weeks after seeding, and at flowering stage, a full rates of Centurion (clethodim) and Axial (pinoxaden) were sprayed to achieve weed control. In all environments, at maturity, the desiccant Reglone (diquat) was applied to dry pea plots before harvesting.

3.2.3 Weather and plant measurement

Major weather variables including temperature, rainfall and relative humidity were recorded from automatic weather stations (Coastal Environmental Systems, Seattle, USA) established at each study site and year, and vapor pressure deficit was determined from air temperature and relative humidity (Jensen et al., 1990). Weather sensors were logged every fifteen minutes and data sets were summarized for daily readings. The daily maximum air temperature and rainfall accumulation during the growing season are presented in Figure 3.1, and a summary of vapor pressure deficit, relative humidity and air temperature specific to flowering and pod set stages (most sensitive stages to heat stress) is presented in Table 3.2.

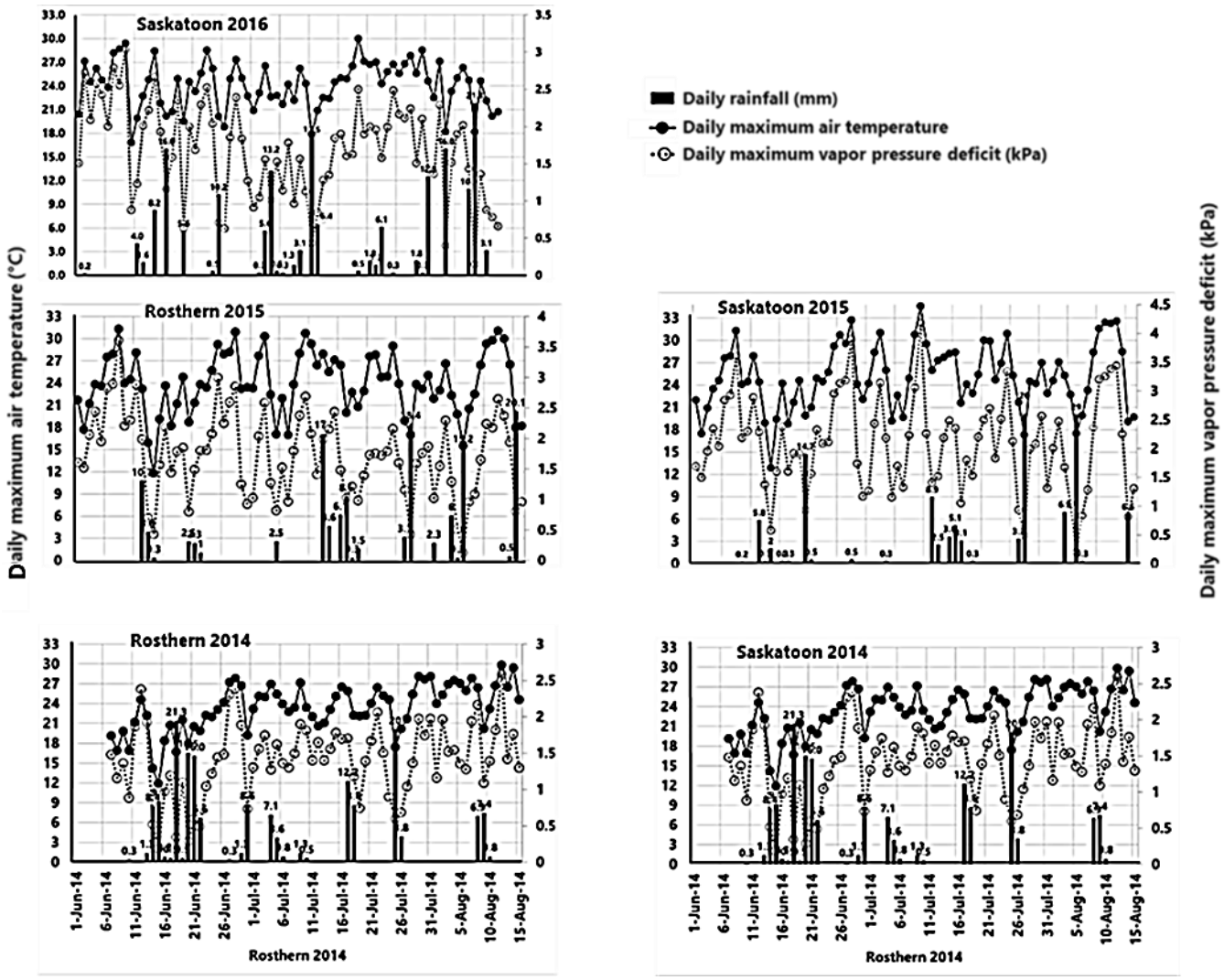


Figure 3.1. Daily maximum air temperature, vapor pressure deficit and rainfall distribution during the growth duration of pea at Rosthern and Saskatoon (2014-2016).

Canopy temperature (CT) was measured using a hand held infrared thermometer (Model 6110.4ZL, Everest Interscience Inc, Tucson, AZ, USA). The infrared (IR) thermometer can measure a temperature range -40 ℃ to 100 ℃ with accuracy of ± 0.5 ℃. Canopy temperature (CT) was measured 5 to 8 times in each location in a season during the reproductive growth whenever the air temperature was above 25 ℃ within 3 hours centred solar noon when the pea was assumed to experience maximum transpiration rate. The IR thermometer was held on the canopy surface for about 10 seconds within 15° field of view so that wider canopy could be accounted, avoiding the soil view from the lenses. During the 10 seconds the thermometer accounts a range of measurements and stabilize on a mean value. Chlorophyll content was estimated using a SPAD502Plus chlorophyll meter (Spectrum technologies Inc, Aurora, IL. USA). A SPAD reading

of < 35 indicated a yellow green canopy, and a readng > 50 indicated a dark green canopy. Similarly, the SPAD reading was taken 4 to 5 times in a season on three leaves per plot at a time) on fully expanded leaf at second or third node counting down from the tip. The average values of CT and SPAD over a season per plot were used for analysis. Flowering duration, the time duration in days from when 50% of the plants in a plot had an open flower to when 50% of the plants had terminated flowering was recorded.

Measurements at physiological maturity (when 80% of pods in the plots turned to brown) included: plant height (vine length from the soil level to the tip of the main stem), reproductive stem length (vine length from the first flowering node to the tip of the main stem), stem thickness between second and third rodes from the tip, reproductive node numbers (nodes counted from the first flowering node to the tip of the main stem), pod numbers (total pods with at least one viable seed on the main stem), and pod to node ratio (ratio of pod numbers to reprdoctive node numbers on the main stem) were determined. For each growth and reproductive variable, three plants per plot were sampled at random, and the mean value was used for analysis. Lodging was scored at physiological maturity using a 1-9 scale according to Zhang et al (2006) where 1 was upright and 9 was fully flat. Finally, each plot was separately harvested and seed yield was determined in gram per unit area.

3.2.4 Data analysis

Statistical analysis on canopy temperature, chlorophyll content by SPAD meter, pod number, seed yield, reproducvtive nodes, pod to node ratio, flowering duration, plant height, and reproductive stem length was performed using the mixed procedure of SAS (Version 9.4, SAS Institute). Before performing analysis of variance (ANOVA), normal distribution of residuals and homogeneity of variance were checked. Then ANOVA, with the least square difference (LSD) test ($P < 0.05$), was performed. The effects of environment, cultivar and environment x cultivar interaction were treated as fixed effects and blocks nested in environment was considered as a random effect. The DDFM = Kenwardroger option was considered for approximating the degrees of freedom for means for unbalanced data. The treatment structure within cultivars, such as canopy color, leaf type, canopy habit, and flower color were compared using contrast statements in the procedure GLM. Traits used for the contrast test were canopy color (dark-green vs. bright green), leaf type (semileafless vs. normal), and plant habit (upright vs. vining), flower color (colored vs.

white) and within colored flowers (red vs. pink) for all traits. Pearson correlation coefficients (r) and their significance levels were determined for $P < 0.05$. A principal component analysis was performed using data analysis function of XLSTAT package of Microsoft Excel, by using the means of phenotypic traits to infer overall association among traits and cultivars.

3.3 Results

3.3.1 Weather characteristics

The most heat and drought stress situation was observed in environment S15, where the daily maximum average air temperature was 27.0 ℃, 2.21 kPa VPD, nine days of air temperature above 28 ℃, and low cumulative rainfall (23 mm) during the reproductive growth (Table 3.2). In contrast, SN16 was the least stressed environment with 23.4 ºC maximum average air temperature, 1.42 kPa VPD, only one day with a daily maximum air temperature above 28 ℃, and 59 mm of rainfall during the reproductive growth. R15 was also relatively hot and dry with a daily maximum average air temperature of 25.4 °C, 1.75 kPa VPD, seven days daily maximum temperature was above 28 °C, and 38 mm of rainfall. S16 was moderately hot and wet with a daily maximum average air temperature of 24.8 ℃, three days above 28 ℃, and 52 mm rainfall. R14 was moderately warm and wet with a daily maximum average air temperature of 24 ℃, one day above 28 ℃, and 62 mm rainfall. S14 was moderately hot and dry with a daily maximum average air temperature was 24.6 °C, 1.81 kPa VPD, four days above 28 ℃, and 38 mm of rainfall during reproductive growth. Daily VPD depends on the daily ambient air temperature and relative humidity and there was significant positive association with the VPD and air temperature (Figure 3.2).

Table 3.2. Experiments and weather summary of the six environments (two locations, and three years, 2014-2016) during the pea flowering and pod setting stages. N = 24 days of flowering duration. ± Standard deviation, VPD: vapor pressure deficit.

Environments	Seeding date	Weather variables during flowering and pod set stages							
		Rainfall (mm)	Daily max mean air temperature (℃)	Daily maxumum mean VPD (kPa)	Number of days with maximum air temperature (°C)				
					≤ 20	≥ 25	≥ 28	≥ 31	≥ 34
R14	Late	62	24.1 ± 2.46	1.45 ± 0.47	2	8	2	0	0
S14	Late	39	24.8 ± 2.75	1.81 ± 0.58	1	9	4	0	0
R15	Late	38	25.4 ± 3.21	1.75 ± 0.47	2	13	7	1	0
S15	Late	23	27.1 ± 3.39	2.22 ± 0.77	1	16	9	3	1
S16	Late	52	25.0 ± 2.69	1.69 ± 0.54	1	17	3	0	0
SN16	Normal	59	23.2 ± 2.74	1.43 ± 0.59	3	8	1	0	0

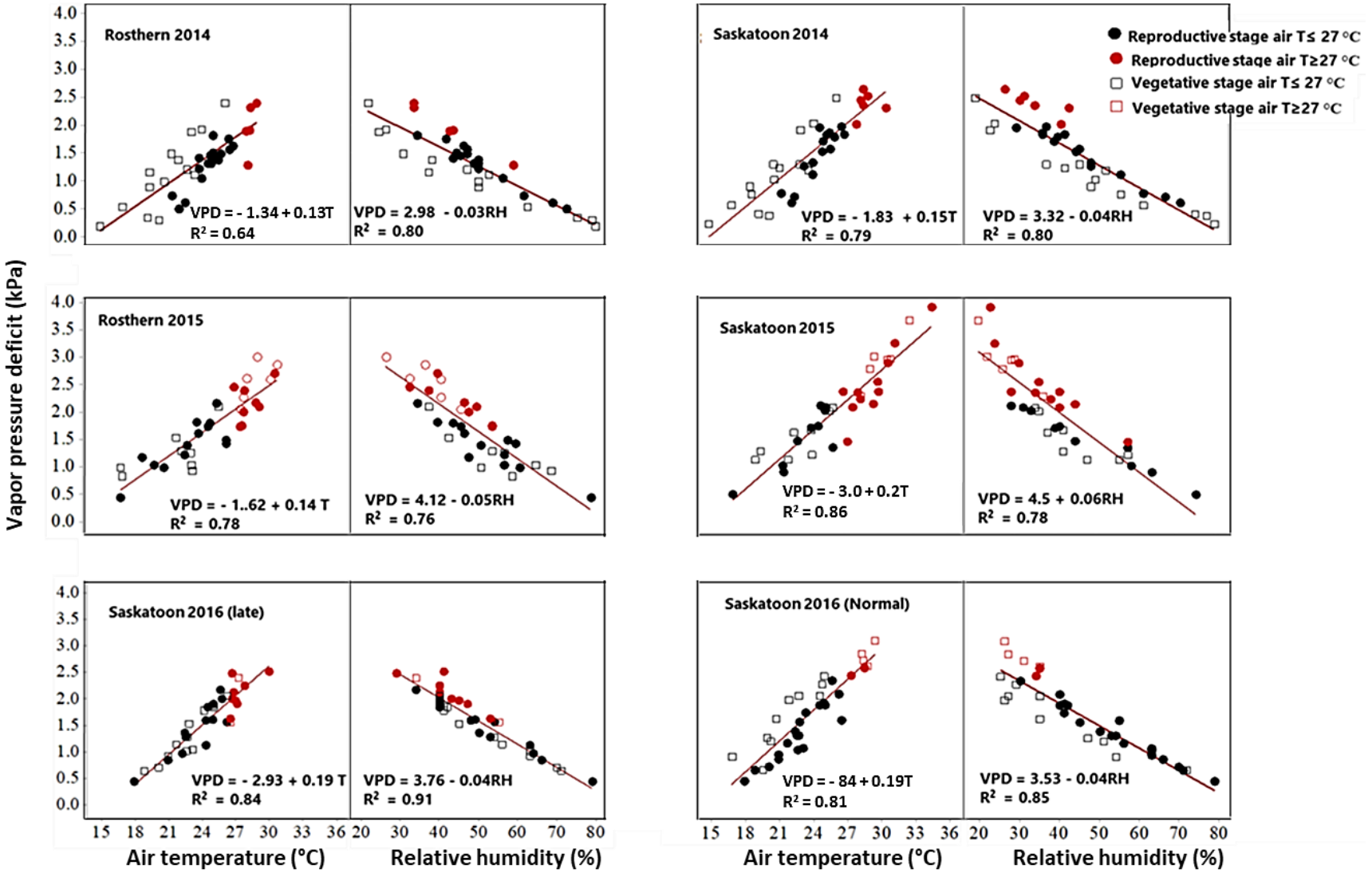


Figure 3.2. Daily maximum vapor pressure deficit relationship with daily maximum ambient air temperature and relative humidity across the six environments during the late vegetative and reproductive stages (N=24 to 32 days). 27 ℃ was the threshold temperature beyond which the pea cultivars face heat stress.

3.3.2 Effects of environment and cultivar

Analysis of variance showed environment (E) and cultivar (C) main effects were significant ($P < 0.001$) for canopy temperature, SPAD leaf greenness, lodging score, stem thickness, reproductive stem length, internode length, plant height, flowering duration, number of reproductive nodes, pod number, pod to node ratio, and seed yield. The C x E interaction was non-significant for all traits except for canopy temperature, flowering duration, pod number, pod to node ratio, and seed yield (Table 3.3).

3.3.3 Canopy temperature

Canopy temperature increased as the weather became hot and dry with high VPD in S15 and R15 environments; and decreased with low air temperature and VPD in SN16 (Table 3.2). Year 2015 was hot and dry, which increased CT and VPD, respectively by 2 °C and 0.43 kPa compared with 2016 (Table 3.2). Canopy temperature was positively correlated with air temperature ($r = 0.71$, Figure 3.4) and the correlation was negative with rainfall ($r = 0.81$, Figure 3.5). Contrast analysis revealed cultivars with the normal leaf and vining habit had significantly higher CT than cultivars with the semileafless leaf and upright habit (Table 3.5).

Cultivars with lower CT (< 26 °C) included Kaspa, 03H267-04HO2006, CDC Golden, CDC Vienna, Mini, TMP 15206, TMP 15181, TMP 15202, Delta and CDC Meadow. In contrast, cultivars with higher CT (> 26.3°C) were TMP 15116, Naparnyk, Torsdag, MPG87, TMP 15213, Rally, and TMP 15179 (Table 3.3). The average CT of cultivars grown under environments S15 and R15 ranged from 26.6-27.7 °C, where cultivars CDC Vienna, Kaspa, TMP 15202, MFR043, CDC Golden, MPG87, Delta, and CDC Meadow were cooler at 26.6-27 °C; cultivars Superscout, TMP 15206, TMP 15181, Rally, Torsdag, Naparnyk, TMP 15213, and TMP 15179 were warmer at 27.3-27.7 °C. Under lower air temperatures prevalent at S16 and SN16, the average CT ranged from 24.8-25.6 °C. Cultivars Kaspa, Delta, 03H267-04HO2006, TMP 15202, Naparnyk, Aragorn, Eclipse, 03H107P-04HO2026, and TMP 15206 were cooler (24.8-25.1 °C) than cultivars CDC Sage, MFR043, TMP 15213, Mini, and Superscout (25.5 °C).

3.3.4 Lodging

Across the six environments, the average lodging score ranged from 4.0 to 4.9 in the 1 to 9 scale. Environments S14 and R14, relatively cool and wet, were associated with a higher lodging

score and SN16 had the lowest lodging score. Cultivars CDC Meadow, CDC Golden, Kaspa, CDC Sage, Eclipse, and Delta had < 3.3 lodging scores, whereas cultivars TMP 15116, Naparnyk, Superscout, and Rally had lodging scores of > 5.8. Cultivars with the vining habit, normal leaf, and bright-green canopy color lodged more on average than cultivars with the upright habit, semileafless leaf, and dark-green canopy color (Table 3.5). Lodging was also positively correlated with canopy temperature ($r=0.73$, $P < 0.001$), reproductive stem length ($r = 0.48$, $P < 0.05$) and internode length ($r = 0.52$, $P < 0.05$; Table 3.4.).

3.3.5 Plant growth

Maximum plant growth measured by stem thickness and length was attained from the relatively hot and wet environment of S16, and minimum growth was associated with the dry and hot environment of S15. Across the six environments, stem thickness ranged from 3.0 to 4.1 mm. Generally, moderately hot and wet environments encouraged plant growth, whereas dry and hot environments diminished the overall growth-related performance. Cultivars Mini, MPG87, TMP 15179, Delta, CDC Golden, and CDC Sage had narrower stems (2.89 to 3.26 mm), whereas Rally, TMP 15181, Superscout, Kaspa, Naparnyk and MFR043 had wider (3.65 to 4.25 mm) stems. Stem thickness was not influenced by leaf type. However, cultivars with the vining canopy habit had wider stems than upright cultivars. The average plant height, reproductive stem length, and internode length of cultivars Torsdag, TMP 15181, 40-10, Naparnyk, TMP 15206 and TMP 15116 was 126.7, 57.2, and 6.7 cm respectively. Cultivars Mini, Rally, MPG87, and Superscout were shorter with an average plant height of 53.3 cm, reproductive stem length of 27.8 cm, and internode length of 4.0 cm. Plant height and reproductive stem length had significant positive correlation with pod number, reproductive nodes, lodging, flowering duration and canopy temperature (Table 3.4.)

3.3.6 Flowering duration

Across the six environments, mean flowering duration varied from 15 to 28 days; environments S15 and SN16 were associated with a shorter and a longer flowering duration, respectively. As the average daily maximum air temperature increased from 23.4 ºC in SN16 to 26.9 ºC in S15, the corresponding flowering duration decreased by 5 days, from 23 to 18 days (Table 3.3). Under the relatively cool and wet environment SN16, the average flowering duration

of cultivars TMP 15206, TMP 15181, TMP 15116, Delta, TMP 15202, and TMP 15179 was 25.5 days; However, in environment S15, the flowering duration of these cultivars was decreased by 4 days. Flowering duration of CDC Meadow, Superscout, Aragorn, 03H107P-04HO2026, and Kaspa decreased by 6 days. Flowering duration was positively correlated with the number of reproductive nodes ($r = 0.82$, $P < 0.001$), pod number ($r = 0.57$, $P < 0.001$), reproductive stem length ($r = 0.80$, $P < 0.001$), and total vine length ($r = 0.65$, $P < 0.001$), so a longer flowering duration increased overall pea growth and yield (Table 3.4.). Generally, cultivars with a bright-green canopy color, vining canopy habit and normal leaf type flowered significantly longer than cultivars with a dark-green canopy color, upright canopy habit, and the semileafless leaf (Table 3.5).

3.3.7 Reproductive node number

Across the six environments, the number of main stem reproductive nodes ranged from 6.5 to 8.7. Compared with environment SN16, number of reproductive nodes was reduced by 21% as weather became hot and dry in S15 (Table 3.3). Cultivars TMP 15179, TMP 15116, TMP 15181, TMP 15206, Torsdag, and MFR043 had 9 or more reproductive nodes per plant. Rally, Superscout, KASPA, 03H267-04HO2006, Eclipse and MPG87 had 7 or fewer reproductive nodes per plant. The cultivars developed by the CDC (CDC Golden, CDC Meadow, CDC Sage and CDC Vienna) produced 7 to 8 reproductive nodes. Contrast analysis demonstrated that cultivars with a vining habit and bright-green canopy color had more reproductive nodes than cultivars with an upright habit and dark-green canopy color (Table 3.5).

3.3.8 Pod number, pod to node ratio, and seed yield

Compared with SN16, pod number in S15 was reduced by 40% which indicated pod set was one of the most affected traits by heat stresses (Table 3.3). However, there was remarkable variation among cultivars in pod number across environments. Under the most stressed environment (S15), cultivars CDC Meadow, MPG87, CDC Vienna, Delta, CDC Golden, and MFR043 had 7.5 to 8.5 pods per plant, and under SN16, cultivars TMP 15181, Torsdag, TMP 15116, TMP 15206, TMP 15213, MFR043, Naparnyk, TMP 15179, TMP 15202, and CDC Golden had 11 to 13 pods per plant. Environment SN16 also had the highest pod to node ratio whereas R14 and S14 were associated with the smallest pod to node ratio. The pod to node ratio was < 1

under R14 and S14 for 54% and 70% of cultivars, respectively. Under S15, cultivars Delta, CDC Meadow, Superscout and Aragorn had 1.24 to 1.37 pods per node, but cultivars 40-10, TMP 15206, Rally, TMP 15181, TMP 15116, CDC Sage and Mini had < 1 pod per node. Cultivars with the semileafless leaf type and upright canopy habit had a greater pod to node ratio than cultivars with the normal leaf type and vining canopy habit, demonstrating that normal leaf and vining plants aborted more flowers and pods (Table 3.5). Compared with the non-stressed environment (SN16), the average seed yield of the heat stressed environments (R14, R15, S15 and S16) was decreased by 24%. Under heat stress condition, cultivars CDC Meadow, TMP15213, CDC Golden and Delta had > 300 g m^{-2} whereas cultivars MPG87, Superscout, Mini and Rally had < 200 g m^{-2} (Table 3.3, and Figure 3.8).

Table 3.3. Means of canopy temperature, lodging score, and various growth and yield traits of the 24 pea cultivars grown across six environments, and probabilities from analysis of variance (ANOVA) showing effects of environment, cultivar, and environment x cultivar interaction on the traits. Means with a common letter within each column under each trait were not different at $P \leq 0.05$. N = 96 for environment, and N = 24 for cultivar averaged over 4 reps.

Effects	Canopy temperature (ºC)	Lodging Score (1–9)	Reproductive stem length (cm)	Internode Length (cm)	Plant height (cm)	Flowering duration (days)	Node numbers	Pod numbers	Pod to node ratio	Seed Yield (g m^{-2})
Environments										
R14	25.9 ± 0.29c	4.6 ± 1.4ab				20.8 ± 2.6c	8.7 ± 1.0a	8.6 ± 1.5bc	1.0 ± 0.14cd	227 ± 6.5d
S14	26.5 ± 0.2b	4.9 ± 1.8a				21.2 ± 1.6bc	8.4 ± 0.9a	7.9 ± 1.7cd	1.0 ± 0.15d	
R15	27.2 ± 0.28a	4.3 ± 1.5bc	31 ± 8c	4.5 ± 1.5c	81 ± 14c	19 ± 2.4d	7.0 ± 1.1b	7.4 ± 1.3d	1.1 ± 0.15b	299 ± 8.9b
S15	27.0 ± 0.4a	4.3 ± 1.4bc	27 ± 6c	4.1 ± 1.4c	82 ± 11c	18.1 ± 1.5e	6.5 ± 1.0b	6.6 ± 1.2e	1.0 ± 0.19bc	243 ± 6.1d
S16	25.2 ± 0.4d	4.2 ± 1.5bc	49 ± 10a	5.7 ± 1.4a	114 ± 17a	21.7 ± 1.0b	8.7 ± 1.1a	9.1 ± 1.4b	1.2 ± 0.15b	269 ± 7.4c
SN16	25.2 ± 0.34d	4.0 ± 1.4c	43 ± 9b	5.1 ± 0.9b	101 ± 18b	23.2 ± 1.0a	8.5 ± 1.3a	11 ± 1.3a	1.3 ± 0.10a	342 ±8.9a
Cultivars										
03H107P-04HO2026	26.2 ± 0.8c-h	3.6 ± 1.1j-k	31 ± 6.3h-j	4.3 ± 1d-h	93 ± 14e-g	18 ± 3lm	7.5 ± 1.4h-k	8.1 ± 2.1e-g	1.1 ± 0.12d-g	296 ± 14f-h
03H267-04HO2006	26.1 ± 0.7f-i	3.6 ± 0.9j-k	34 ± 6.9gh	4.9 ± 0.9b-d	94 ± 18ef	20 ± 2g-j	7.1 ± 1.2kl	7.6 ± 1.9g-i	1.1 ± 0.15d-g	303 ± 12e-g
40-10	26.2 ± 0.6c-h	5.3 ± 0.9cd	43 ± 7.4e	5.3 ± 1bc	126 ± 11bc	21 ± 2e-g	8.3 ± 1.7e-g	7.1 ± 1.9ij	0.8 ± 0.11k	252 ± 28k
Aragorn	26.2 ± 0.8d-h	3.6 ± 1.1j-k	33 ± 5.9hi	4.4 ± 0.8d-g	83 ± 14ij	19 ± 3k-m	7.8 ± 1.4g-i	8.8 ± 1.6c-e	1.2 ± 0.13cd	270 ± 29i-k
CDC Golden	25.9 ± 0.7ij	2.8 ± 0.8mn	29 ± 5.4i-k	4.0 ± 0.8g-i	81 ± 8i-k	20 ± 3h-j	7.7 ± 1.2g-j	8.6 ± 2.2c-f	1.2 ± 0.22cd	360 ± 18a
CDC Meadow	26.0 ± 0.7g-j	2.6 ± 0.8n	29 ± 5.4jk	3.9 ± 0.9g-i	85 ± 15hi	20 ± 3h-j	7.4 ± 1.1h-k	9.1 ± 1.9b-d	1.3 ± 0.15ab	355 ± 26ab
CDC Sage	26.3 ± 0.7c-g	3.0 ± 1.0l-n	30 ± 7.6i-k	4.1 ± 1.3f-i	87 ± 13g-i	20 ± 3i-k	7.9 ± 1.6f-h	6.9 ± 2ij	0.9 ± 0.19jk	263 ± 12i-k
CDC Vienna	25.9 ± 0.6ij	3.4 ± 0.9j-l	31 ± 5.8h-j	4.1 ± 0.8f-i	76 ± 9kl	21 ± 2e-h	7.9 ± 1.4f-h	8.7 ± 1.5c-f	1.2 ± 0.12de	284 ± 28g-i
Delta	26.1 ± 0.8g-j	3.3 ± 0.9k-m	29 ± 6.4jk	4 ± 0.9f-i	90 ± 14f-h	21 ± 3f-h	7.5 ± 1.1h-k	8.7 ± 1.2c-e	1.2 ± 0.15bc	331 ± 20b-d
Eclipse	26.1 ± 0.7e-i	3.0 ± 1l-n	37 ± 9.4fg	5.4 ± 1.4b	91 ± 15e-h	22 ± 2e-g	7.1 ± 1.2j-l	7.6 ± 2g-i	1.1 ± 0.2d-g	313 ± 23d-f
KASPA	25.8 ± 0.7j	3.0 ± 0.8l-n	31 ± 5.4h-j	4.7 ± 0.9c-f	96 ± 16ef	18 ± 3m	7.0 ± 1.4kl	8 ± 1.9f-h	1.2 ± 0.16cd	321 ± 19c-e
MFR043	25.9 ± 0.5ij	3.8 ± 0.8h-j	32 ± 7.5h-j	3.6 ± 0.7ij	95 ± 13ef	21 ± 2e-h	9.3 ± 1.5bc	9.3 ± 2.3a-c	1 ± 0.17g-i	257 ± 17jk
Mini	26.3 ± 0.6c-f	5.5 ± 1.1c	24 ± 6.2 l	3.7 ± 1.2h-j	71 ± 19l	19 ± 2j-l	7.1 ± 1.4j-l	7.3 ± 2hi	1.1 ± 0.17f-i	168 ± 13m
MPG87	25.9 ± 0.7ij	4.0 ± 1h-i	27 ± 6.5kl	3.3 ± 0.9j	61 ± 10m	21 ± 2e-g	8.6 ± 1.6de	8.4 ± 1.3d-g	1.0 ± 0.14hi	219 ± 23l
Naparnyk	26.6 ± 0.8ab	6.1 ± 0.7b	51 ± 10d	7.0 ± 1.7a	129 ± 19b	21 ± 2e-h	7.3 ± 1.6i-l	9.3 ± 2.5a-c	1.4 ± 0.12a	277 ± 12h-k
Rally	26.7 ± 0.7a	6.8 ± 1.2a	31 ± 8.9h-j	5.1 ± 1.2bc	63 ± 14m	19 ± 2k-m	6.3 ± 1.2m	6.4 ± 1.8j	1.0 ± 0.18g-i	141 ± 23n
Superscout	26.3 ± 0.7b-e	6.3 ± 1.2b	29 ± 7.9i-k	4.2 ± 1e-h	78 ± 20jk	18 ± 3m	6.8 ± 1.2lm	7.3 ± 1.7hi	1.2 ± 0.17d-f	170 ± 12m
TMP 15116	26.3 ± 0.7c-f	5.8 ± 0.7bc	63 ± 12.5b	7.0 ± 1.3a	136 ± 16a	23 ± 2bc	9.1 ± 1.6b-d	8.8 ± 2.7c-e	0.9 ± 0.18ij	282 ± 18g-j

TMP 15179	26.4 ± 0.9bc	4.4 ± 1.2fg	30 ± 7.5i-k	3.6 ± 0.9h-j	82 ± 21i-k	22 ± 3de	8.7 ± 1.5c-e	9.1 ± 2b-d	1.1 ± 0.16e-h	267 ± 14i-k
TMP 15181	26.1 ± 0.8d-i	5.5 ± 0.7c	59 ± 9.4c	6.6 ± 1.1a	120 ± 13cd	24 ± 3b	9.6 ± 1.6ab	9.9 ± 2.6a	1.0 ± 0.14f-i	311 ± 12d-f
TMP 15202	26.0 ± 0.7h-j	4.8 ± 1.2ef	39 ± 9.0f	4.8 ± 1b-e	97 ± 19e	22 ± 3ef	8.2 ± 1.5e-g	8.1 ± 2e-g	1.0 ± 0.15hi	251 ± 30k
TMP 15206	26.2 ± 0.9c-h	4.9 ± 1.1de	69 ± 11.3a	7.2 ± 1a	133 ± 18ab	26 ± 2a	9.9 ± 1.3a	9.8 ± 2.4ab	1.0 ± 0.12i	314 ± 21d-f
TMP 15213	26.4 ± 0.9b-d	4.2 ± 1.1gh	34 ± 9.4gh	4.4 ± 1.1d-g	95 ± 15ef	20 ± 2g-i	8.4 ± 1.6ef	10 ± 2.7a	1.3 ± 0.15a-c	343 ± 27a-c
Torsdag	26.3 ± 0.8c-f	5.8 ± 1.4hc	58 ± 8.5c	6.9 ± 1.8a	116 ± 19d	23 ± 2cd	8.7 ± 1.6de	9.6 ± 2.7ab	1.1 ± 0.14d-f	278 ± 15g-j
P Values										
Cultivar	***	***	***	***	***	***	***	***	***	***
Environment	***	***	***	***	***	***	***	***	***	***
Cultivar x environment	*	ns	ns	ns	ns	*	*	*	*	***

***, **, *Significant at P ≤ 0.001, 0.01 and 0.05, respectively; ns = non-significant at P ≤ 0.01. .± Standard error

Table 3.4. Correlation test of various physiological, growth and yield traits, number of reproductive nodes, pod number, flowering duration, SPAD reading, canopy temperature, pod to node ratio, reproductive internode length, reproductive length, and plant height of 24 pea cultivars grown under field condition across six environments; N = 24, averaged over four replications per environment.

Trait variables	RN	Pods	Lodging	FD	SPAD	CT	PNR	IL	RL
Reproductive node numbers (RN)									
Pod numbers (Pods)	0.68**								
Lodging (1-9)	0.72***	-0.07							
Flowering duration (FD)	0.81***	0.57**	0.23						
SPAD	-0.46*	-0.22	-0.22	-0.60**					
Canopy temperature (CT)	-0.15	-0.08	0.73***	-0.03	-0.20				
Pod to node ratio (PNR)	-0.37	0.42*	-0.25	-0.29	0.30	0.01			
Internode length (IL)	0.33	0.31	0.52*	0.63**	-0.72***	0.40	-0.08		
Reproductive stem length (RL)	0.62**	0.48*	0.48*	0.80**	-0.74***	0.26	-0.20	0.94***	
Plant height (PH)	0.54**	0.31	0.31	0.65**	-0.80***	0.17	-0.12	0.84***	0.88***

***, **, * Significant correlation at $P \leq 0.001$, 0.01 and 0.05, respectively. ns = non-significant at $P \leq 0.05$.

3.3.9 Principal component analysis

Principal component analysis (PCA) based on the correlation of canopy and leaf traits revealed a clear differentiation of cultivars based on their leaf and canopy traits and heat stress response. The first two principal components (PCs) explained 79% of the total variability in the data (Figure 3.3). Canopy temperature (CT) positioned in distant (obtuse to straight angle) from pod number and pod to node ratio, indicating a significant negative correlation between canopy temperature and pod formation traits (Figure 3.3), which agreed with the negative association obtained through correlation analysis (Table 3.4.). Likewise, growth and reproductive traits including reproductive stem length, plant height, and flowering duration, were positioned near to each other to the positive side of PC1 indicating their positive phenotypic association.

The two PCs biplot (Figure 3.3) also revealed four distinct clusters of cultivars as indicated by color shaded regions (purple, red, blue and green) based on leaf and canopy traits, and the cultivars' heat stress response. Almost all cultivars with semileafless leaves and upright canopies under heat stress (shaded purple) were positioned in the negative side of PC1, opposite to the cluster of cultivars with normal leaves and vining growth habits under non-heat stressed conditions (shaded blue). Also, cultivars with the normal leaf type and vining canopy under heat stress (shaded red) positioned in the positive side of PC2, at an obtuse angle with semileafless and upright cultivars under non-heat stressed conditions (shaded green). These results implied that under heat stress, pea cultivars with the normal leaf and a vining canopy habit were more sensitive to heat stress (Figure 3.3), as they were positioned at nearly 180^{o} (negative correlation) to yield related traits including pod number and pod to node ratio. In contrast, under non-heat stress, these cultivars were superior in growth related traits including plant height and reproductive stem length (Figure 3.3). Further details of how the 24 cultivars responded to the different growth conditions and how they positioned on the PCA are presented in appendix E.

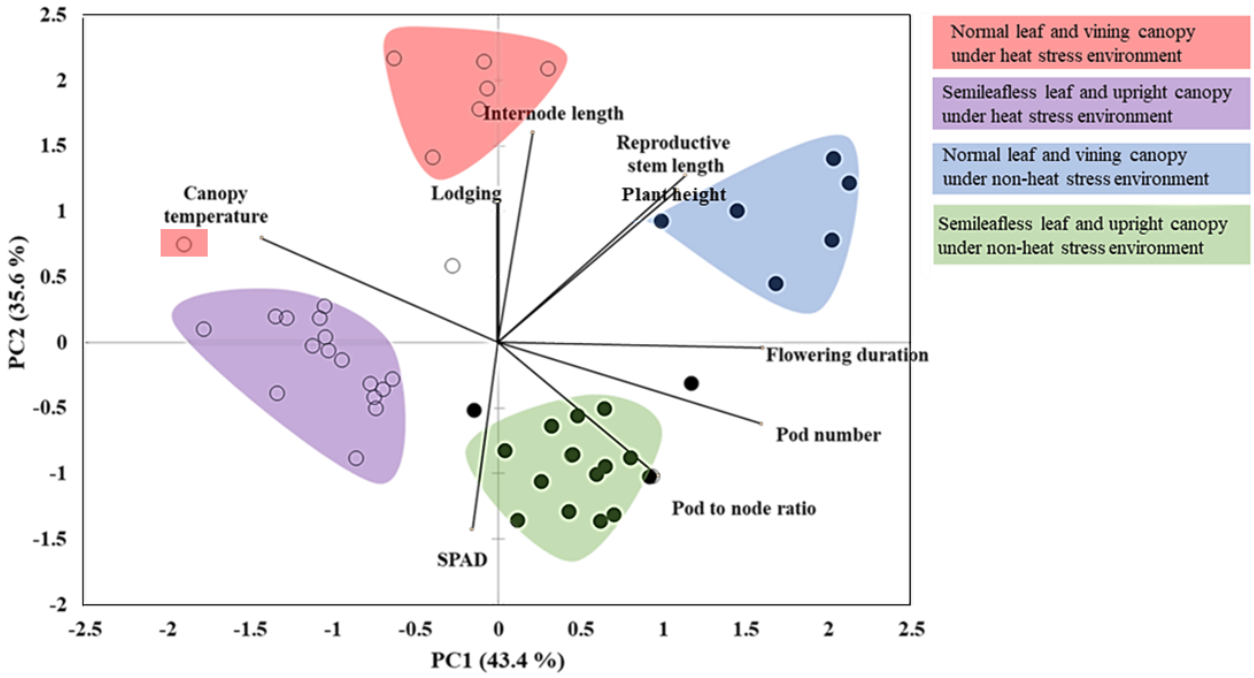


Figure 3.3. Bi-plot from principal components demonstrating relationship between number of reproductive nodes, pod number, pod to node ratio, chlorophyll content estimation by SPAD meter (SPAD), canopy temperature, lodging score, reproductive stem length, internode length, plant height, and flowering duration of pea cultivars grown under heat stressed (Saskatoon 2015 late seeding date, empty circles) and non-heat stressed (Saskatoon 2016 normal seeding date, full circles) environments. The PCA showed four clear clustering among the 24 cultivars under the two environments, and the clustering was primarily caused by the leaf type and canopy habit response to the heat stress. Eigenvalue proportions of the first two components are marked as a percentage on the axis label. Each symbol is a cultivar averaged over four replications per environment.

3.4 Discussion

3.4.1 Cultivar response under heat stress

Surprisingly, pea grown under cool conditions (SN16) did not show the greatest performance in growth-related parameters, namely stem thickness, plant height and reproductive stem length. Pea grown in S16 was superior in all growth-related measurements, demonstrating that a moderately hotter temperature and sufficient rainfall encouraged vegetative growth, but not necessarily resulted in greater economic yield. Environments S15 and R15, where the heat stress occurred concurrently with drought resulted in poorest growth. My results corroborate research where heat stress and other weather variables controlled overall plant growth and aboveground biomass of pea (Poggio et al., 2005; Sadras et al., 2013). Main reproductive stem length was

associated with internode length, and I noted that heat stress shortened internode length of the reproductive stem. However, the heat-stress can be confounded with water deficit stress in the field, because heat and water stress frequently occur together (Hall, 2004; Bueckert et al., 2015) and water deficiency also reduces internode length (Klepper et al., 1971).

Although research reports are inconsistent with respect to the threshold temperature for heat stress in pea, reports agree that the most critical stage is from flowering through pod filling (Jeuffroy et al., 1990; Guilioni et al., 1997; Jiang et al., 2015). Pea yield is a function of plant population density, pod number per plant, seed number per pod, and weight of individual seeds (French 1990; Poggio et al., 2005). My results revealed that pod set was the trait most affected by heat stress of those I measured (Table 3). A 40% reduction in pod set was seen between the most stressed (S15) and least stressed (SN16) environments. Such a reduction was associated with flower and pod abortion due to heat stress, detrimentally impacting pea yield (French 1990; Jeuffroy et al., 1990; Guilioni et al., 1997). Pod set relies on functioning male and female floral components, both of which are reportedly very sensitive to heat stress (Jiang et al., 2015; Wang et al., 2006).

Pea cultivars demonstrated a remarkable variation in seed yield and pod number across environments. Under the most stressed environment (S15), cultivars MFR043, CDC Golden, Delta, MPG87, CDC Vienna, and CDC Meadow had 7.5-8.5 pods per plant. Interestingly, these cultivars also had a high seed yield, pod number per node (2 pods per node is the maximum potential in most pea genotypes) and a cooler canopy temperature. The most obvious visual characteristics of these cultivars were upright canopy habit and semileafless leaf, traits which were associated with lower canopy temperature, and therefore contributed to heat tolerance (Alvino and Leone, 1993; Baigorri et al., 1999). Cultivars with an upright canopy habit avoided direct contact with the hot soil surface, and the upright canopy allowed enhanced aeration within the canopy, to contribute to a cooler CT on hot days (Alvino and Leone, 1993; Heath and Hebblethwaite, 1985). An upright canopy is more erectophile, so light radiation is reflected off surfaces to a greater extent (Heath and Hebblethwaite 1987). The stipule is smaller in overall lamina size, and leaves have more tendrils and petiole all of which likely help conserve water by decreasing transpiration loss (Reicosky et al., 1980; Wilson et al., 1981; Kashiwagi et al., 2008). In contrast, under the least-stress conditions at SN16, cultivars MFR043, TMP 15213, TMP 15206, TMP 15116, Torsdag, and TMP 15181 had a superior yield of > 12 pods per plant. Most of these cultivars had a normal leaf,

indeterminate growth habit with vining, a large leaf area and greater performance in growth-related traits, suggesting these cultivars have high yield potential under non-stress conditions through a longer reproductive phase (Bueckert et al., 2015); a similar result was observed in chickpea (Berger et al., 2006).

The flowering duration was highly dependent on environmental variables, particularly temperature and moisture (Guilioni et al., 2003; Bueckert et al., 2015). My results revealed a strong negative correlation (r = -0.88***, Figure 3.4c) between air temperature and flowering duration, and a positive correlation (r = 0.78***, Figure 3.5c) between cumulative rainfall and flowering duration (Figure 3.5), Both results have been linked high temperature and low moisture to a reduced reproductive phase and lower yield in pea (Guilioni et al., 1997; Sadras et al., 2012; Bueckert et al., 2015). High temperature also induced acceleration of phenology and reduced yield in chickpea (Wang et al., 2006), and cultivars with capacity to resist heat and maintain yield generally had long flowering phases (Berger et al., 2006).

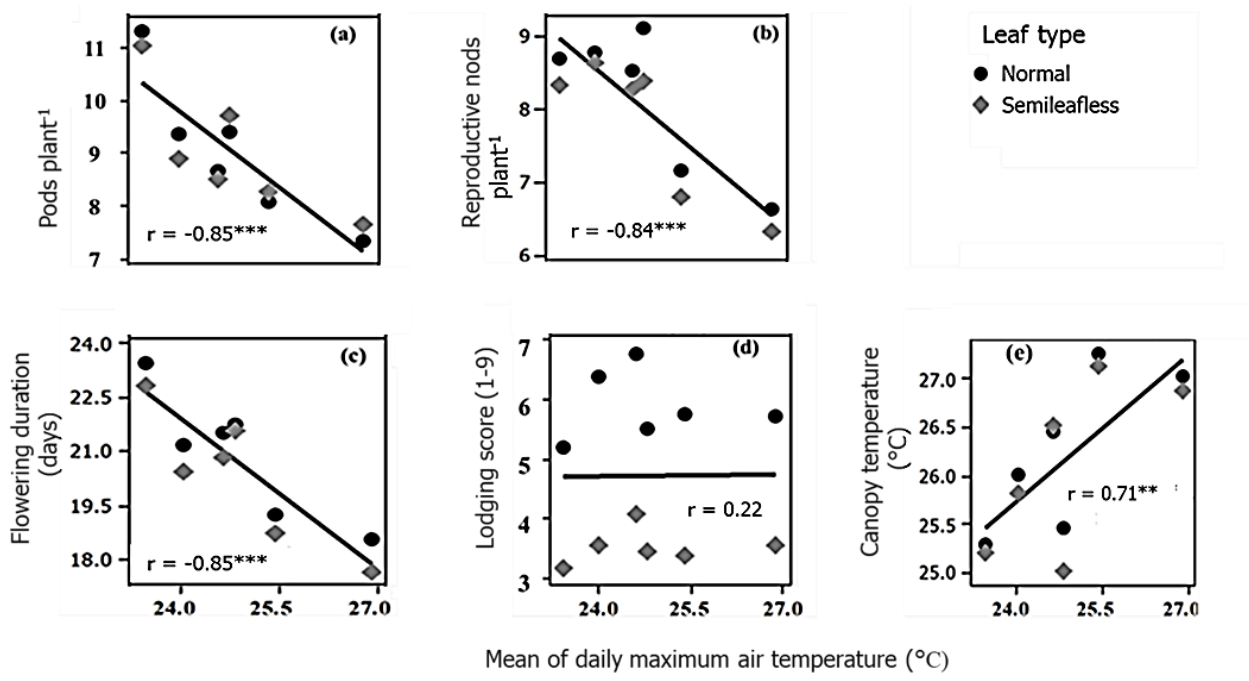


Figure 3.4. Relationship between mean daily maximum air temperature during reproductive stage and growth, phenology and yield traits: pod number per plant (a), number of reproductive nodes per plant (b), flowering duration (c), lodging score (d), and canopy temperature. A significant negative relationship was observed between daily maximum air temperature and number of reproductive nodes, pods and flowering duration; the relationship was positive for canopy temperature. The values presented in the y-axis of each panel are mean values per environment per leaf type, N = 48 (12 cultivars per leaf type, and four replications).

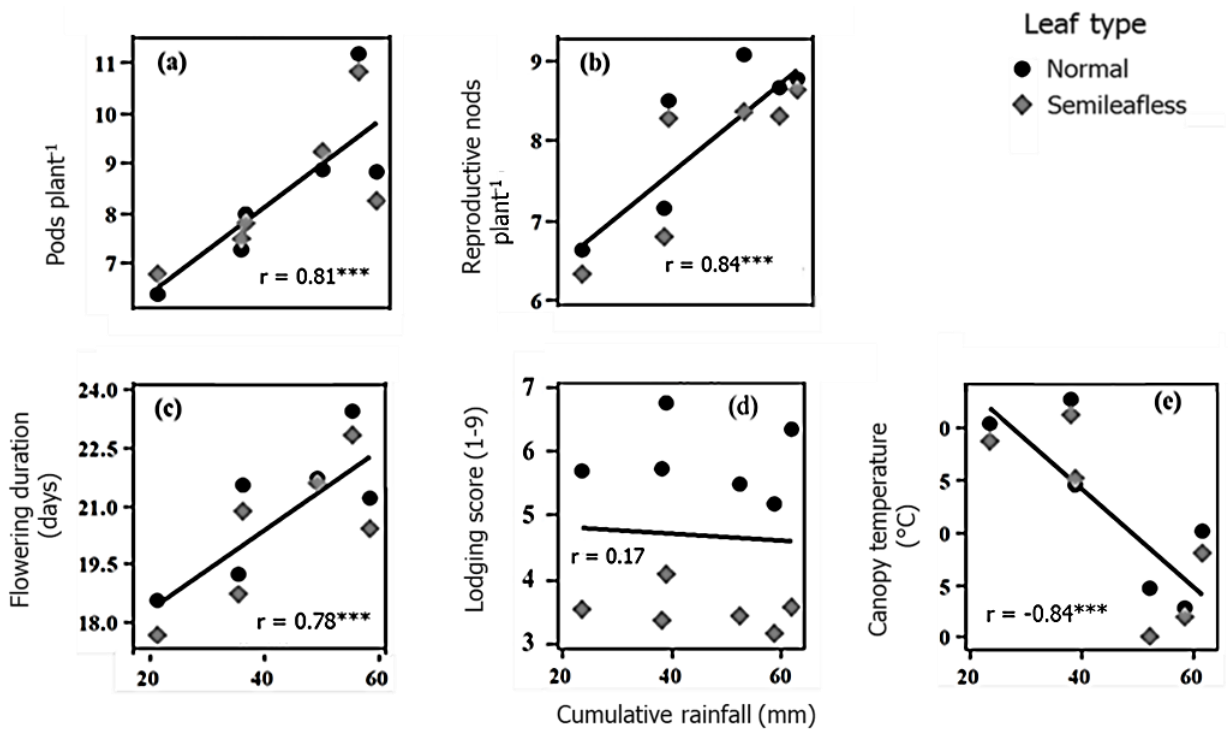


Figure 3.5. Relationship of cumulative rainfall during flowering stage with growth and yield traits: number of reproductive nodes per plant (a), pod number per plant (b), pods to node ratio (c), flowering duration (d), lodging score (e), and canopy temperature. A significant positive correlation was observed between rainfall and number of nodes, pod number and flowering duration; and the relationship was negative for canopy temperature. The values presented in the y-axis of each panel are mean values per environment per leaf type, N = 48 (12 cultivars per leaf type, and four replications).

Table 3.5. Contrast analysis to determine plant characteristics effects on number of reproductive nodes (RN), pod number per plant (Pods), flowering duration (FD), node to pod ratio (NPR), main reproductive stem length (RL), plant height (PH), stem thickness (SD), lodging score (1-9), and canopy temperature (CT) of 24 pea cultivars grown in field in across six environments in western Canada. Bright-green (N = 240), Dark-green (N = 336), Normal leaf (N = 288), Semileafless (N = 288), Upright (N = 336), Vining (N = 240), Colored (N = 144), White (N = 432).

Contrasts	Levels	CT (°C)	SPAD	Lodging score (1-9)	Growth parameters			Reproductive parameters			
					SD (mm)	RL (cm)	PH (cm)	RN (Num)	Pods (Num)	NPR	FD (Days)
Canopy color											
	Bright-green	26.20	44.0	4.6***	3.61***	44***	107***	8.2***	8.8***	1.08 ns	21.3***
	Dark-green	26.16	46.5***	4.2	3.35	33	87	7.8	8.1	1.06	20.2
Leaf type	Normal	26.3*	45.0	5.3***	3.52 ns	41***	99***	8.1 ns	8.5 ns	1.04 ns	21***
	Semileafless	26.1	45.9*	3.4	3.39	35	91	7.8	8.4	1.09.	20.4
Plant habit											
	Upright	26.1	46.5***	3.5	3.42	32	89	7.8	8.5 ns	1.09*	20.1
	Vining	26.3*	43.9	5.6***	3.51***	46***	103***	8.2*	8.4	1.03	21.4***
Flower color											
	Colored	26.10	45.4 ns	3.8	3.45 ns	35	97*	7.7	8.2	1.08 ns	19.8
	White	26.21	45.5	4.6***	3.46	39***	95	8.1***	8.5*	1.06	20.9***

***, **, *Significant at P ≤ 0.001, 0.01 and 0.05, respectively. ns = non-significant at P ≤ 0.01.

3.4.2 Canopy contributed to heat tolerance

My results demonstrated that canopy traits and environmental factors influenced canopy temperature (Table 3). In S15, cultivars with semileafless leaves and the upright habit had significantly higher pod number and pod to node ratio than cultivars with normal leaves and vining canopy (Figure 3.7a and b). The greater pod to node ratio of semileafless cultivars under S15 was associated with decreased flower and pod abortion percentages. An ephemeral life cycle is a major drought escape mechanism (Fischer and Maurer 1978; Bueckert and Clarke 2013), and a shortened vegetative phase, early flowering and completion of the plant lifecycle before the onset of terminal heat stress is perceived as a heat escape strategy (Hasanuzzaman et al., 2013). Interestingly, under the heat stressed environment S15, the relatively longer flowering duration of cultivars possessing normal leaves and the vining habit did not result in a yield advantage over the semileafless cultivars (Figure 3.7a and c), likely due to ovule and pod abortions (Baigorri et al., 1999).

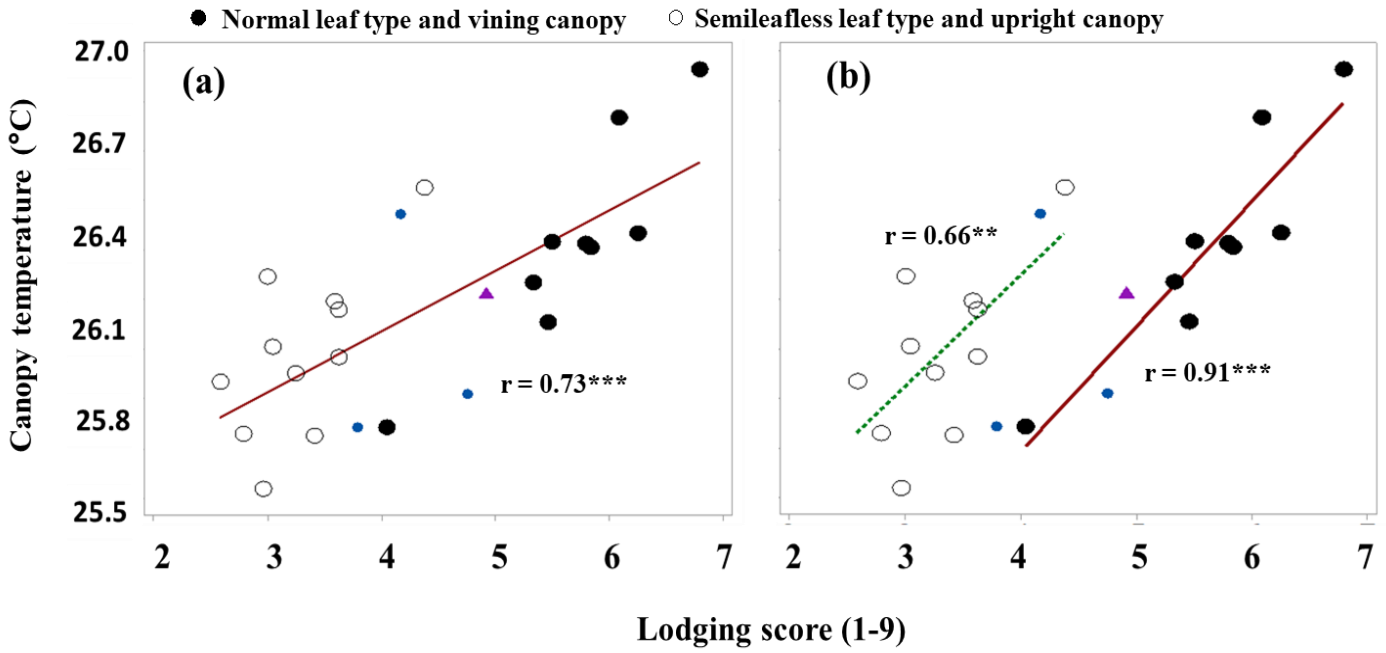


Figure 3.6. Lodging (1-9: 1 is completely upright and 9 is completely flat) and canopy temperature correlations of pea grown in field condition across six environments in western Canada. Each symbol represented a cultivar's mean of 24 repetitions (six environments by four replications). The full black circles are cultivars with vining canopy habit and normal leaf type; and the empty black circles are cultivars with upright canopy habit and semileafless leaf type. In panel a, lodging was correlated with all cultivars whereas in panel b lodging and CT correlation was done separately by leaf types.

A strong positive correlation ($r = 0.73$, $P < 0.01$) was observed between lodging and canopy temperature (Figure 3.6), demonstrating that lodging exacerbated heat stress on the crop (Alvino and Leone, 1993). Under field conditions, lodged cultivars make direct contact with the hot soil surface, from which heat can transfer to the plant canopy as conducted heat (Flerchinger et al., 2003). Lodged canopies are planophile, and absorb more heat from the sun and reflect less, and this further results in a hotter canopy. On hot days, when air temperature > 27 °C, the soil surface temperature was roughly 15 ℃ higher than canopy temperature (our unpublished data). Cultivars with high lodging scores (5 - 7) were 40-10, TMP 15181, Mini, Torsdag, TMP 15116, Naparnyk, Superscout, and Rally, and all these had normal leaves, and a vining canopy habit except for TMP 15181. In contrast, all cultivars with lower lodging scores (2.6 - 3.5) namely CDC Meadow, CDC Golden, Kaspa, CDC Sage, Eclipse, Delta, CDC Vienna, 03H107P-04HO2026, 03H267-04HO2006, and Aragorn were semileafless and upright in plant habit (Alvino and Leone, 1993).

The question is which plant traits are most closely associated with, and drive a higher canopy temperature? Traits associated with lodging and growth habit were strongly associated with CT. The two correlations in panel b of Figure 3.6, and appendix D of this thesis demonstrated that cultivars with vining plant habit and normal leaf type had warmer canopy temperature than cultivars with the upright plant habit and semileafless leaves. Most semileafless cultivars had relatively larger petioles and a high density of stiff tendrils that increase interplant locking and support, so plants remained upright (Heath and Hebblethwaite 1985). Upright plants were able to avoid direct contact with the hot soil surface, and canopy cooling could be from a condition of less heat absorption, more radiation and heat reflection, and enhanced air flow through the canopy. Cultivars with a normal leaf type had bigger lamina size, which is usually associated with high heat load on plant canopy and low heat dissipation due to limited air flow through the canopy, and therefore leads to a higher canopy temperature (Alvino and Leone, 1993; De Boeck et al., 2016). My unpublished data showed about 33-40% of the area of semileafless leaves (stipules, petioles and tendrils) is made up of petiole and tendrils. Petiole and tendril, being cylindrical, long and of narrow diameter, may help reduce heat absorption and increase reflection (Heath and Hebblethwaite 1985). Most semileafless cultivars are relatively shorter plants with less lamina area and greater photosynthetic efficiency compared with the normal leaf cultivars (Heath and Hebblethwaite 1987). In a study conducted under moisture stress, semileafless pea cultivars used water more efficiently under drier conditions, with an advantage of photosynthetic contribution

from tendrils (Wilson et al., 1981). Moreover, semileafless cultivars often have better resistance against disease and pest attacks (Snoad 1974), which usually contribute to higher canopy temperature. These features and qualities make semileafless pea cultivars more tolerant to heat stress over cultivars with the normal leaf.

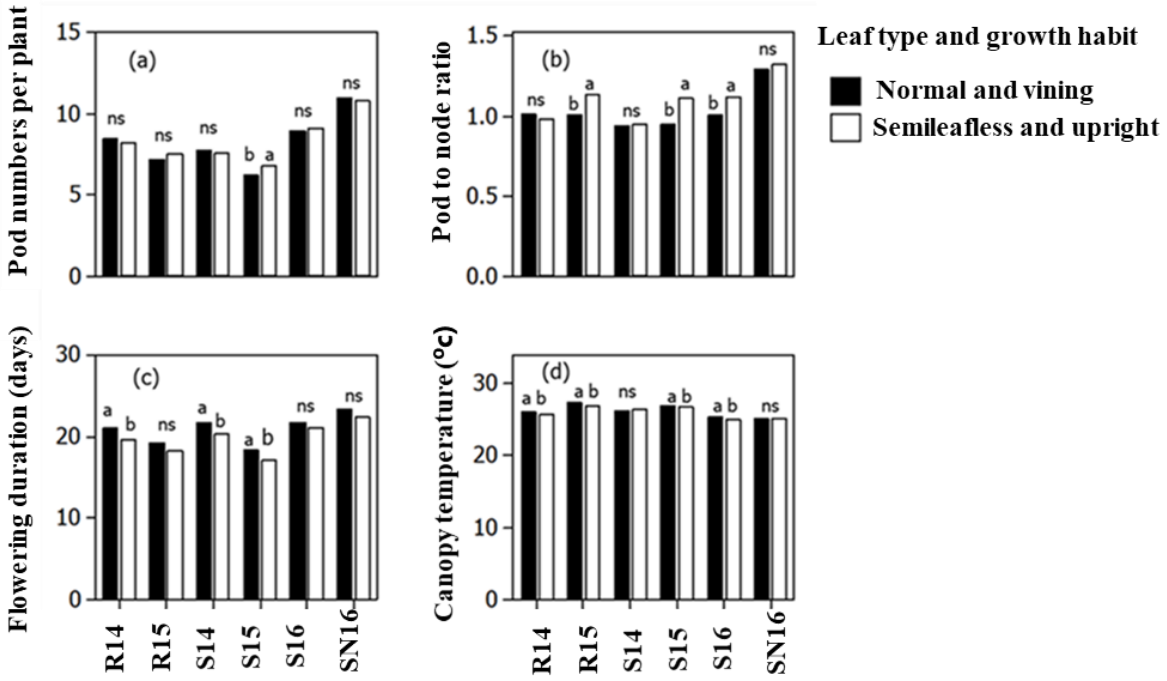


Figure 3.7. Effect of leaf type and plant growth habit on number of pods per plant (a), pod to node ratio (b), flowering duration (c), and canopy temperature (d) of pea grown across six environments (R14, R15, S14, S15, S16 and SN16). Bars with similar letters within each panel are not significantly different at P (< 0.05). Each bar represented an overall mean per environment per leaf type/growth habit, N = 48 (12 cultivar per leaf type, four times replicated).

Apart from the plant factors, environmental factors such as air temperature and rainfall amount and distribution have obvious influences on canopy temperature (De Boeck et al., 2016). A significant positive correlation ($r = 0.71$, $P < 0.01$) was observed between air and canopy temperature (Figure 3.4e). My result demonstrated that high VPD, which enhance crop evaporative demand, was associated with the warmer environments and lead to a higher canopy temperature. High VPD leads to heat stress by enhancing water loss which in turn leads to a high canopy temperature due to the inability of plants to cool themselves. In a separate study (data not shown

here) I noticed that pea cultivars with normal leaf had lower leaf water content than the cultivars with semileafless leaf. In water deficit stress, heat stress was aggravated by the inability of the plant to effectively reduce canopy temperature through transpirational cooling (Kashiwagi et al., 2008). Based on results of a heat and moisture stress study on soybean, Reicosky et al. (1980) concluded that high radiation and moisture stress contributed to increased canopy temperature on hot days. Environments with higher cumulative rainfall were associated with lower canopy temperature indicating maintained optimal soil moisture, and presumably maintained crop evapotranspiration, did not disrupt crop metabolism (Kashiwagi et al., 2008)

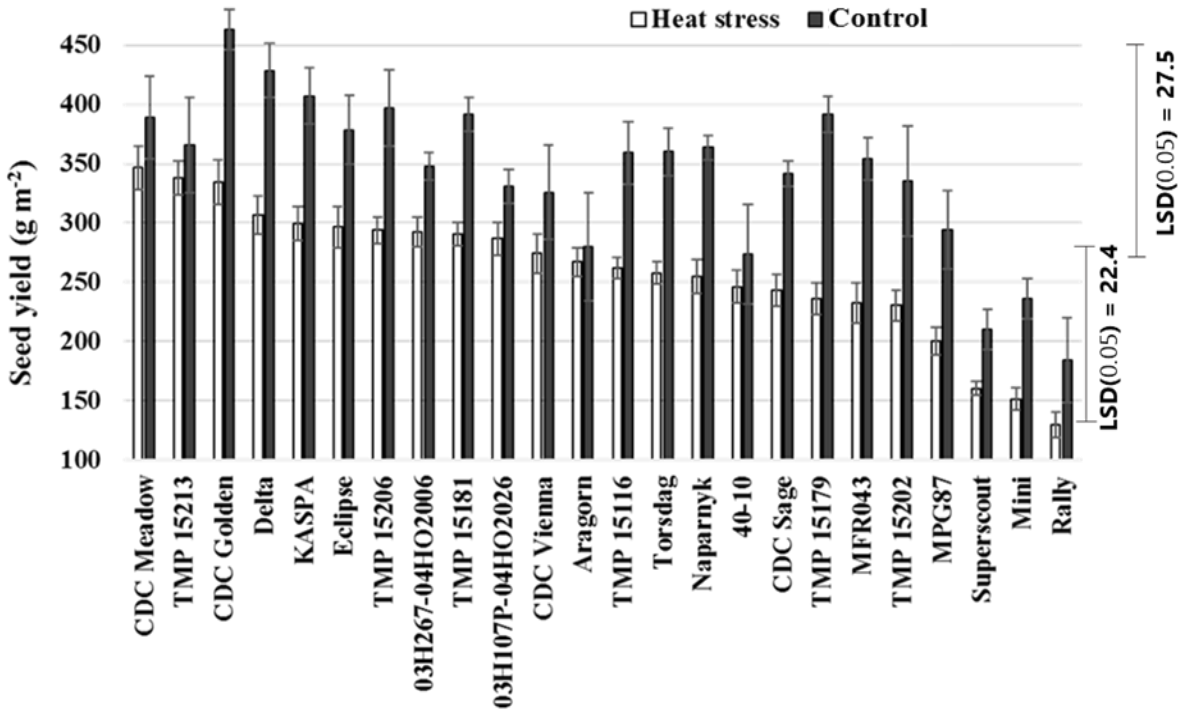


Figure 3.8. Cultivars average seed yield under normal and heat stressed environments. Each bar is the cultivar's mean yield averaged over four replication and four environments (N = 16) for heat stressed, and N = 4 for control environment. Error bars are standard error of mean.

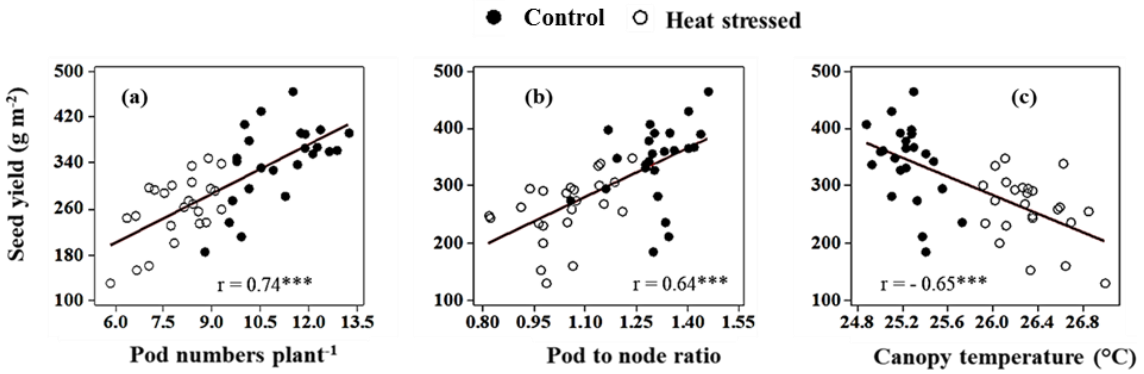


Figure 3.9. Seed yield correlation with number of pods per plant (a), pod to node ratio (b), and canopy temperature (c). Each full black circles in each panel represent an average value (N = 4) of a cultivar under normal growth condition whereas the empty circle is an average value (N = 16) of a cultivar under the heat stressed environments.

3.5 Conclusions

Most previous heat stress studies on pea concentrated on reproductive abortion, reduced lifecycle length and yield. In addition to these measures, I found that pea leaf and canopy traits played a key role in heat stress tolerance. Remarkable variation was observed among pea cultivars for traits related to the adaptation to heat stress. Under heat stressed environments, cultivars with the semileafless leaf, determinate growth habit, upright canopy habit, and a dark-green canopy color maintained a cooler canopy and demonstrated improved yield performance including seed yield, pod number per node and per plant. Semileafless cultivars had less lamina which may help to conserve moisture, but the corresponding increase in tendrils helped plants cling together and maintain an upright habit. Cultivars with an upright canopy minimized heat absorption, and enhanced aeration through the canopy to help with canopy cooling. On the other hand, plants with high lodging score had more heat absorption due to greater attenuation of solar radiation, and thus they are conducting the heat from the upper surface into the short lodged canopy. Likely, the warmer canopy temperature for the lodged cultivars was due to increased plant conduction of heat, plants' canopy storage of heat, and increased heat convection over the canopy surface. The role of pigments in leaves and the canopy requires additional research, but I found intensity of greenness was associated with a cooler canopy. Generally, cultivars were better suited to heat stress in the field if they possessed semileafless leaves, and an upright growth habit. In contrast, under optimal

environments, the normal leaf type, indeterminate and vining canopy habit had a greater potential for growth-related traits and more pods in the growing season.

Transition section between Chapter 3 and Chapter 4

Effects of heat stress on canopy temperature, growth and productivity, and roles of plant architecture and leaf type as heat tolerance traits were elucidated in Chapter 3. Under heat stressed environments, cultivars with the semileafless leaf, determinate growth habit, and upright canopy habit maintained a cooler canopy and demonstrated improved heat avoidance and yield performance. In contrast, under optimal environment, the normal leaf type, indeterminate and vining canopy habit had greater potential for growth-related traits and more pods in a growing season. The aim of the next experiment in Chapter 4 was to investigate the influence of heat stress on pigments and wax and their role as heat tolerance traits and to examine their association with leaf spectral reflectance and various vegetation indices.

CHAPTER 4. LEAF PIGMENTS AND WAX AS HEAT TOLERANCE TRAITS; AND THEIR ASSOCIATION WITH SPECTRAL VEGETATIVE INDICES IN PEA

4.1 Introduction

Pea (*Pisum sativum* L.) is a widely grown pulse crops for its nutritious seeds and soil fertility benefits (Cousin 1997; McPhee et al., 2003; Dahl et al., 2012) but production is hampered by heat stress which impairs photosynthesis, shortens the crop life cycle, leads to abortions of flowers and pods, and thus to yield loss (Gulilioni et al., 1997; Porter 2005; Bueckert et al., 2015; Huang et al., 2017). Pea heat stress arises when air temperature rises above a threshold during the growing season, or when heat shock occurs if the temperature rise ≥34 ℃ for several hours during sensitive stages (Ridge and Pye, 1985; Bueckert et al., 2015, Huang et al., 2017). Although pea heat sensitivity varies with phenology, heat stress can affect plant performance from germination to maturity (Guiloni et al., 1997; Pumphrey and Ramig, 1990).

To cope with the heat or other environmental stress, plants have developed various physiological and biochemical modifications as avoidance or tolerance strategies (Shepherd and Griffith, 2006; Wahid et al., 2008; Hasanuzzaman et al., 2013). The strategies can broadly be categorized either as a long-term evolutionary modification to phenological and morphological patterns, or a short-term heat avoidance such as transpiration cooling and excess radiation reflection and heat dissipation (Powles, 1984; Havaux, 1989; Wahid et al., 2007). Spectral reflectance in the UV and infrared regions helps to avoid/minimize radiation and heat load (Havaux, 1989; Gamon and Surfus, 1999; Holmes and Keiller, 2002; Shepherd and Griffith, 2006). Epicuticular waxes form an outermost barrier over plant surfaces and contribute to plant survival under stressful environments (Jenks et al., 2000; Willick et al., 2017). In pea, epicuticular wax reduces residual transpiration and thus minimizes excess water loss which helps to maintain tissue water status under drought stress (Sánchez et al., 2001; Hasanuzzaman et al., 2017).

Similarly, pigments may contribute to heat tolerance in many ways such as heat dissipation and protection of vital plant processes (Powles, 1984; Havaux, 1989). For example, Sanchez et al (2001) reported leaf color as a trait that improved crop productivity under drought stress. In barley, greater grain yield was associated with light green and dark green leaves under heat stressed and non-heat stressed conditions respectively (Ceccarelli, 1987). Generally, stay-green, a trait that

delays plant senescence improves yield under drought and heat stress conditions in wheat (Kumar et al., 2010).

Plant spectral reflectance is governed by leaf surface properties, leaf internal structure, and by the concentration and distribution of biochemical components (Filella, et al., 1995; Jacquemoud and Ustin 2001). Spectral reflectance has been effective as a proxy to quantitatively estimate traits associated with biomass, pigment abundance, and leaf water status (Peñuelas et al., 1995; Ollinger, 2011; El-Hendawy et al., 2017). Although sufficient availability of epicuticular wax on leaf surfaces has been reported as a drought tolerance trait in several crops (Sanchez et al., 2001; Shepherd and Griffiths, 2006; Guo et al., 2016; Willick et al., 2017), its contribution as a heat tolerance trait is rarely reported. Similarly, leaf pigments and their association with heat tolerance or avoidance in pea was not addressed sufficiently. Here, I hypothesized that leaf wax and pigment concentrations enhance pea heat resistance by avoidance in pea, and these biochemical factors are distributed across the pea germplasm. The specific objectives of the study were 1) to investigate roles of wax and leaf pigments on heat response of pea, 2) to investigate the association of pigments and wax with spectral vegetative indices, and 3) to determine leaf wax and pigment distribution among 24 pea cultivars.

4.2 Materials and Methods

A field study was conducted for three years (2014-2016) at two locations Rosthern (52º66'N, 106º33'W) and Saskatoon (52º12'N, 106º63'W), across six environments in western Canada. The environments, plant materials, experimental design, plant growth management, and weather condition were exactly as described in section 3.2.2 of this thesis.

4.2.1 Leaf sample collection and area determination

The pea cultivars used in this study had normal and semileafless leaves. Cultivars with the normal leaf had a wider flat leaf surface from stipules and leaflets and a relatively smaller petiole size, and cultivars with the semileafless leaf had larger petioles with tendrils, stipules but they lacked leaflets. Unless otherwise stated, throughout this chapter the flat leaf surface and the stalk are referred to as 'lamina' and 'petiole', respectively. For chlorophyll, carotenoid, anthocyanin and wax measurements, fully developed young leaves sampled typically from the second or third main stem node counting down from the apical tip, were collected twice during the pea growing season, at early flowering, and flower termination. The leaf samples were separated into the lamina and

petiole components, and separately scanned using winRHIZO (Regent Instruments Inc, Quebec City, Canada) to determine their respective projected surface areas.

4.2.2 Bulk wax measurement

Wax extraction and quantification were done according to Sanchez et al (2001), originally adopted from Ebercon et al., (1977). Wax extraction from the lamina and petiole samples were performed by rinsing samples into 10 ml chloroform for 15 sec at room temperature in tubes. The extract was evaporated to dryness in a water bath at 70 °C. Then 5 ml acidic $K_2Cr_2O_7$ (prepared from sulfuric acid and potassium dichromate; 20g $K_2Cr_2O_7$ per L of H_2SO_4) was added into the tubes containing wax and boiled at 100 °C in a water bath for 30 min. After cooling, 5 ml distilled water was added, and the spectral absorbance was measured at 590 nm using an Agilent 8453 diode array spectrophotometer, wavelength 190–1100 nm, 1.6±0.5 nm resolution equipped with Chem Station software for UV-visible spectroscopy (Agilent technologies, Santa Clara, California, USA). From the absorbance peak, the wax concentration was calculated using equation developed from a standard curve that was developed from a linear ($R^2 > 0.98$) relationship of a series of known beeswax concentrations, at 590 nm. More information of the wax extraction and quantification procedure is presented in the appendix A.

4.2.3 Chlorophyll and carotenoid measurements

Chlorophyll measurement was performed according to Lichtenthaler (1987). A 1.22 cm^2 stipule disc, and the entire petiole (with a known projected area) were used for the chlorophyll extraction. The samples were placed in 5 ml glass vials with a tight cap and 3 ml of 100% acetone was added and kept for 6 hours at room temperature for complete extraction. Then, the samples were homogenized by vortex and centrifuged for 5 min at 5000 rpm. The clear supernatant solution was used for absorbance measurement using the spectrophotometer at wavelengths of 470, 645, 662 and 710 nm. Further detail can be found in the appendix B of this thesis. Concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoid were determined in µg cm^{-2} according to Lichtenthaler (1987) using the following equations:

Chlorophyll a (µg ml^{-1}) = [11.24(A662–A_{710})]–[2.04(A645–A_{710})] ----------4.1

Chlorophyll b (µg ml^{-1}) = [20.13(A645–A_{710})]–[4.19(A662–A_{710})] ----------4.2

Total Chlorophyll (µg ml^{-1}) = [7.05(A662–A$_{710}$)] + [18.09(A645–A$_{710}$)] ----------4.3

Total carotenoid (µg ml^{-1}) = [1000A470–A$_{710}$)]–[1.90 Chlorophyll a]–[63.14 Chlorophyll b] --------4.4

4.2.4 Anthocyanin measurements

A 1.33 cm^2 disc cut from stipule, and the entire petiole were used for the anthocyanin extraction. Total anthocyanin in the leaf samples was determined using the spectrophotometric method previously described by Abdel-Aal and Hucl (1999). The samples were placed in 5 ml glass vials and 3 ml of acidified ethanol (85:15) v/v ratio using 95% ethanol and 1.428N HCl, respectively at pH 1, and vials were capped. Samples were kept in the solution overnight at room temperature for complete extraction. Then, the samples were mixed by vortex and centrifuged for 5 min at 5000 rpm. The clear supernatant solution was used the absorbance reading at 535 and 663 nm using the spectrophotometer. Total anthocyanin concentrations in µg cm^{-2} was calculated according to (Murray and Hackett, 1991) as:

Anthocyanin (µg ml^{-1}) = A535-0.24(A663) ----------4.5

The 535 nm and 663 nm are the wavelength peaks for anthocyanin and chlorophyll a absorbance, respectively under the above extraction conditions. Further detail can be found in the appendix C of this thesis

4.2.5 Spectral reflectance and vegetative indices

Spectral reflectance factor measurements on stipules were taken three to five times per plot across the six environments during the reproductive stage using a portable spectroradiometer PSR-1100F (Spectral Evolution Inc, Lawrence, MA, USA), a device enabling hyperspectral readings with a range of 320–1,126 nm, and 1.6 nm resolution, a total of 512 discrete narrow bands. A 1 m fiber-optic cable with industry-standard interfaced with the instrument was supported by a PSR-1100 Pistol Grip to help me specifically direct the sensor to stipules for the spectral measurements. A stipule fully exposed to sun was targeted from a fully developed young leaf either at second or third node counting from the tip of pea main stem. Measurements were taken on cloud-free and usually hot days around solar noon (between 11:00 and 14:00 h) avoiding shadow, cloud, and any other interferences I could possibly control. Before measurements, reflectance was taken on a white plate that provided maximum reflection. The reflectance was measured by holding the fibre

sensor within 3 cm distance from the leaf sample approximately within 80° viewing angle. The reference reflectance was repeatedly taken every 15 min (equivalent to about every 15 plots) to adjust with the changing irradiance from the sun, and more frequently if clouds stopped measurements.

Vegetative and pigment indices including normalized difference vegetation index (NDVI), green normalized difference vegetation index (GNDVI), photochemical reflectance index (PRI), triangular vegetation greenness (TVG), water band index (WBI), anthocyanin reflectance index (ARI), carotenoid reflectance index (CRI), red reflectance percentage (Red), near infrared reflectance percentage (NIR), and normalized pigment and vegetation index (NPCI) were calculated from the reflectance data. The respective equations, bandwidth and center bands, brief proxies of the VI, and references are presented in table 4.1 of this thesis.

Table 4.1. Summary of vegetation indices expression and their major agricultural applications.

Vegetation index	**Equation**	**Major application**	**Reference**
NDVI	$(R_{nir}-R_r)/(R_{nir}+R_r)$	Productivity, vigor, health, greenness	Rouse et al., 1974
GNDVI	$(R_{nir}-R_g)/(R_{nir}+R_g)$	Biomass, vegetation health	Gitelson & Merzlyak, 1994
PRI	$(R_{531}-R_{570})/(R_{531}+R_{570})$	Pigment	Gamon et al., 1997
NPCI	$(R_{680}-R_{430})/(R_{680}+R_{430})$	Pigment	Peñuelas et al., 1994
TVG	$0.5[120(R_{nir}-R_g)-200(R_r-R_g)]$	Pigment	Broge and Leblanc, 2001
ARI	$R_{800}(1/R_{550})-(1/R_{700})$	Pigment	Gitelson et al., 2001
CRI	$1/R_{510})-(1/R_{550})$	Pigment	Gitelson et al., 2002
WBI	R_{900}/R_{970}	Water content	Peñuelas et al., 1993

Where NDVI: Normalized difference vegetation index; GNDVI: Green normalized difference vegetation; PRI: Photochemical reflectance index; NPCI: Normalized pigment and chlorophyll index; TVG: Triangular vegetation greenness; ARI: Anthocyanin reflectance index; CRI: Carotenoid reflectance index; WBI: Water band index; R: reflectance; nir: near infrared band (bandwidth 760−860, center band 820 nm), r: red band (bandwidth 650−700 nm, center band 675 nm); g: green (bandwidth 530−580, center band 555 nm). The center bands were rounded off to nearest whole number (for example 530.5 nm as 531 nm). For VIs calculated from two or more single bands such as ARI, CRI, and WBI, the nearest whole number band was used as the center band (for example 900.6 nm was used as 900 nm, and 969.4 nm was used as 970 nm).

4.2.6 Heat tolerance index

Heat tolerance index (HTI) was determined according to Fernandez (1992) and Fisher and Maurer (1978) with a slight modification to account for pod numbers and pod set ratio, traits most affected by heat stress.

$$\mathbf{HTI} = \frac{(\mathbf{Pod_h})(\mathbf{Pod_c})}{(\mathbf{Pod_{c.ave}})^2} + \frac{(\mathbf{PNR_h})(\mathbf{PNR_c})}{(\mathbf{PNR_{c.ave}})^2} \text{ ----------4.6}$$

Where; Pod_h = pod number under heat stress, Pod_c = pod number under non-heat stress (control), $Pod_{c.ave}$ = grand mean pod number under non-heat stress, PNR_h = pod to node ratio under heat stress, PNR_c = pod to node ratio under non-heat stress (control), $PNR_{c.ave}$ = grand mean pod to node ratio under non-stress condition.

4.2.7 Data analysis

Statistical analysis on chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b, total carotenoid, total anthocyanin, and total wax concentrations from lamina and petiole, canopy temperature, NDVI, GNDVI, PRI, ARI, CRI, NPCI and WBI was performed using the mixed procedure of SAS (Version 9.4, SAS Institute). Then ANOVA with the least square difference (LSD) test ($P < 0.05$) was performed. Environment, blocks and cultivar were used as class variables; the effects of environment, cultivar and environment x cultivar interaction were treated as fixed effects and blocks nested in environment were considered as a random effect. The DDFM = Kenwardroger option was considered for approximating the degrees of freedom for means. The effects of cultivars characteristics including canopy color, leaf type, canopy habit, and flower color were compared using contrast statements in the procedure GLM. Traits used for the contrast test were canopy color (dark-green vs. bright green), leaf type (semileafless vs. normal), and plant habit (upright vs. vining), flower color (colored vs. white) and within colored flowers (red vs. pink) Pearson correlation coefficients (r) and their significance levels were determined for $P < 0.05$. A principal component analysis was performed using the multivariate function of Minitab Statistical Software version 18 (Minitab, 2018), by using each cultivar's mean phenotypic traits to infer overall association among canopy traits and cultivars.

4.3 Results

4.3.1 Effects of environments and cultivars on pigment, wax and VIs

Analysis of variance showed environment (E) and cultivar (C) main effects were significant ($P < 0.0001$) for all the lamina and petiole chlorophyll, carotenoid, anthocyanin and wax concentrations, and for the vegetative indices, NDVI, GNDVI, PRI, ARI, CRI and NPCI. The C x E interaction was also significant ($P < 0.05$) for all traits except for chlorophyll a/b, NDVI and CRI (Table 4.2 and 4.3).

Heat stress led to a significant reduction in lamina and petiole chlorophyll and carotenoid concentrations (Figure 4.2a and 4.2b; and Figure 4.3a and 4.3b). Compared to SN-16 (the non-stressed control), mean lamina chlorophyll a and chlorophyll b concentration in late seeded environments (heat stressed) decreased by 19.4, and 25.5%, respectively. In contrast, the respective chlorophyll a/b ratio, anthocyanin, and bulk wax concentrations were increased by 7.4, 18.7, and 21.6% respectively. The increased chlorophyll a/b ratio under S-15 was due a greater reduction in chlorophyll b concentration than chlorophyll a (Table 4.2). Generally, there was an increasing trend in the leaf lamina and petiole chlorophyll, carotenoid and wax concentrations during reproductive stage, from early flowering to flower termination stages (Figure 4.4). During this period, lamina chlorophyll a, chlorophyll b, carotenoid, and wax concentrations increased by 15.2, 14.8, 5.6, and 39.2% respectively, and the corresponding anthocyanin concentration was decreased by 18.1%. Similarly, the petiole chlorophyll a, chlorophyll b, carotenoid, and wax concentrations were increased by 14.3, 10.2, 6.4, and 52.6% respectively (Figure 4.4). The leaf lamina had 39.6 and 68.2% higher chlorophyll and carotenoid concentrations, respectively than the petiole. In contrast, the petiole had 71.3 and 5.2% higher wax and anthocyanin concentrations, respectively, than the laminas (Table 4.2).

Table 4.2. Mean chlorophyll a, chlorophyll b, chlorophyll a/b ratio, carotenoid, anthocyanin and wax concentrations from pea leaf lamina and petiole of 24 pea cultivars grown across six environments, and probabilities from analysis of variance (ANOVA) showing effects of environment, cultivar, and environment x cultivar interaction on the traits. Means with a common letter within each column under each trait were not different at $P < 0.05$. N = 192 for environment, and N = 48 for cultivar.

	Chlorophyll a (µg cm-2)		Chlorophyll b (µg cm-2)		Chlorophyll a/b		Carotenoid (µg cm-2)		Anthocyanin (µg cm-2)		Wax (µg cm-2)	
Effects	Lamina	Petiole	Lamina	Petiole	Lamina	Petiole	Lamina	Petiole	Lamina	Petiole	Lamina	Petiole
Environment												
R14	28.2 d	15.6 d	8.9 c	5.2 d	3.21 bcd	2.97 a	8.4 a	4.52 bc	1.45 a	1.38 a	32.4 a	56.8 ab
R15	31.7 b	19 c	10.5 b	7.9 c	3.03 cd	2.48 b	8.6 a	5 ab	1.31 ab	1.12 b	28.3 b	58.9 a
S14	26.6 e	17.3 cd	8.3 d	5.7 d	3.27 bc		7.6 b	5.03 a	0.94 c	0.7 c	24.8 c	33.4 c
S15	23.5 f	18.9 c	6.7 e	8 c	3.69 a	2.44 b	5.9 d	4.73 ab	1.26 b	1.3 ab	27.5 b	53.3 b
S16	30 c	21.6 b	9.1 c	9.6 b	3.32 b	2.4 b	7 c	4.18 c	0.88 c	1.17 b	24.2 c	33.7 c
SN16	34.6 a	24 a	11.7 a	11.1 a	2.99 d	2.21 c	8.1 ab	3.57 d	0.95 c	1.46 a	21.4 d	30.7 c
Cultivar												
03H107P-04HO2026	26.8 j-l	18.4 f-i	8.3 i-l	7.5 f-i	3.29 a-f	2.57 de	6.2 k	4.27 e-g	1.15 c-g	1.23 b-e	27.5 f-h	37.4 kl
03H267-04HO2006	28.9 f-h	19.3 d-g	8.9 f-h	8 ef	3.34 a-d	2.56 d-f	6.5 jk	4.72 b-e	1.16 c-f	1.18 b-f	26 hi	37.1 kl
40-10	25.6 lm	19.4 d-g	7.9 kl	7.1 g-j	3.28 c-g	2.79 a-c	6.6 i-k	4.42 d-g	0.99 j	1.04 fg	21.6 mn	44.5 e-j
Aragorn	29.8 d-g	20.6 b-e	9.7 cd	9.3 bc	3.17 e-g	2.42 g-i	6.8 h-k	4.89 b-d	1.13 c-h	1.22 b-e	29.6 b-e	39.6 j-l
CDC_Golden	29.1 e-h	20.1 c-f	9.8 d-f	8.3 d-f	3.13 c-f	2.55 d-g	6.8 h-j	4.59 c-f	1.15 c-g	1.19 b-f	30.6 bc	43.1 f-j
CDC_Meadow	31.6 c-f	19.5 d-g	10.5 c	8.2 ef	3.06 g	2.48 e-i	6.9 g-j	4.6 c-f	1.19 b-d	1.23 b-e	31.3 b	41.2 f-k
CDC_Sage	25.9 k-m	17 hi	8.2 kl	7.7 f-h	3.24 b-f	2.42 f-i	7 g-j	3.95 g	1.1 d-i	1.09 d-g	28.7 c-f	40.2 h-l
CDC Vienna	34.7 b	19.3 d-g	11 b	10.6 a	3.16 e-f	2.1 j	7.1 -i	4.22 e-g	1.29 ab	1.26 a-d	28.8 c-f	40.9 g-l
Delta	29.0 h-j	18.5 f-i	8.5 g-k	8.4 b-f	3.20 c	2.37 hi	7.1 f-i	4.26 e-g	1.08 e-j	1.22 b-e	33.7 a	45 e-i
Eclipse	28.8 g-i	17.8 g-i	8.8 f-j	7.9 fg	3.35 a-d	2.39 hi	7.2 e-i	4.43 d-g	1.2 bc	1.15 b-g	28.1 d-g	39.8 i-l
KASPA	27.3 i-k	17.8 g-i	8.9 f-i	9.3 b	3.2 c-d	2.19 j	7.3 e-h	4.19 e-g	1.18 b-e	1.16 b-g	27.6 f-h	40 h-l
MFR043	31.3 cd	20.9 a-d	9.6 c-e	7.6 f-i	3.21 c	2.8 a-c	7.5 d-g	4.34 e-g	1.18 b-e	1.31 ab	28.1 e-g	44.2 e-j
Mini	29.9 d-g	22.5 ab	9.6 cd	8 ef	3.17 fg	2.83 a	7.5 d-g	5.55 a	1.13 c-h	1.23 b-e	26.4 g-i	54.8 ab
MPG87	31.8 c	22.8 a	10.1 c	8.2 ef	3.22 c-f	2.85 a	7.5 d-g	5.25 ab	1.22 bc	1.42 a	25.1 ij	55.8 a
Naparnyk	24.8 m	16.6 i	7.8 lm	6.3 j	3.19 f-g	2.67 cd	7.6 d-g	3.92 g	1.04 h-j	1.07 e-g	26.2 g-i	45.1 e-h
Rally	29.3 e-h	20 c-f	9.3 d-f	7 g-j	3.26 a-f	2.89 a	7.6 d-g	4.57 c-f	1.07 f-j	1.14 c-g	22.6 lm	53.9 a-c
Superscout	42.5 a	21.8 a-c	15.3 a	9.1 b-d	2.87 h	2.49 e-h	7.8 c-f	5.05 a-c	1.36 a	1.22 b-e	24 j-l	52.4 a-c

TMP15116	23.0 n	18.8 e-h	7.2 m	7.1 g-j	3.29 a-f	2.68 b-d	7.8 c-e	4.17 fg	1.03 h-j	1.17 b-g	19.9 n	50.5 b-d
TMP15179	30.7 c-e	20.5 c-e	10.3 c	8.8 b-e	3.19 e-g	2.46 e-i	8 cd	4.94 b-d	1.2 b-d	1.3 a-c	30 b-d	39.9 i-l
TMP15181	26.5 j-l	21 a-d	8.3 i-l	7.6 f-i	3.27 a-f	2.8 a-c	8.2 bc	4.99 bc	1.05 g-j	1.14 b-g	22.9 k-m	46.4 d-f
TMP15202	27.9 h-j	17.7 g-i	8.4 g-k	6.5 j	3.4 a	2.76 a-c	8.3 bc	4.07 fg	1.05 g-j	1.19 b-f	24.7 i-k	49.1 c-e
TMP15206	26.9 j-l	17.8 g-i	8.3 h-k	8.4 c-f	3.36 a-c	2.34 i	8.8 b	4.1 fg	1.12 c-i	1.15 b-g	24.9 ij	35.9 l
TMP15213	29.2 e-h	18.7 e-h	9 e-g	6.9 h-j	3.35 a-d	2.77 a-c	8.8 b	4.13 fg	1.05 g-j	1.23 b-e	24.9 ij	45.7 d-g
Torsdag	26.4 j-l	18.7 e-h	8.2 j-l	6.8 ij	3.24 b-f	2.8 ab	11.2 a	4.53 c-f	1.02 ij	1 g	21.6 mn	44.8 e-j
Significance												
Environment (E)	***	***	***	***	***	***	***	***	***	***	***	***
Cultivar (C)	***	***	***	***	***	***	***	***	***	**	***	***
E * C	***	***	***	***	ns	***	***	***	***	*	***	***

***, **, *Significant at P ≤ 0.001, 0.01 and 0.05, respectively. ns = non-significant at P ≤ 0.01.

Cultivars Superscout, Rally, CDC Vienna, MPG 87, TMP15179, MFR043, and CDC Meadow had high (> 48 µg cm^{-2}) lamina total chlorophyll concentration under non-stressed, and (> 38 µg cm^{-2}) under heat stressed conditions (Figure 4.2). These cultivars also had high (> 36 µg cm^{-2}) petiole total chlorophyll concentration under non-stressed, and (> 25 µg cm^{-2}) under heat-stressed environments (Figure 4.3). In contrast, TMP15116, Naparnyk, CDC Sage, 40-10, and Torsdag had less (<32 µg cm^{-2}) lamina total chlorophyll concentration under heat stressed (Figure 4.2). Under the heat stressed environment, cultivars with bright green leaves had significantly higher (13.3%) chlorophyll a/b ratio than cultivars with dark green leaves (Table 4.5).

Under the heat stressed environment, cultivars Delta, CDC Meadow, CDC Golden Aragon, and TMP15179 had high (> 31 µg cm^{-2}), and cultivars TMP15116, 40-10, and Torsdag had less (< 24 µg cm^{-2}) lamina wax concentration (Figure 4.2f). Generally under the heat stressed environment, lamina and petiole wax concentrations were negatively correlated to each other, and thus cultivars with high lamina wax were associated with less petiole wax concentration (Figure 4.2f and Figure 4.3f). Cultivars with high (> 50 µg cm^{-2}) petiole wax concentration included MPG87, Mini, Rally, and Superscout (Figure 4.3f). Cultivars with the semileafless leaf had higher lamina wax than cultivars with the normal leaf type (Table 4.5). In contrast, cultivars with the normal leaf had greater petiole wax and chlorophyll a/b ratio than semileafless cultivars (Table 4.5). Under heat stress environment, cultivars with an upright habit had significantly higher lamina wax concentration and chlorophyll a/b ratio than the semileafless cultivars. In contrast, cultivars with a vining growth habit had higher petiole wax concentration and chlorophyll a/b ratio than the upright cultivars (Table 4.5).

4.3.3 Response in reflectance indices

The non-stressed environment SN-16 was associated with higher NDVI, GNDVI, ARI, and CRI and lower RED, NIR and TVG indices. Although values of most vegetation indices were under ‘normal’ ranges for a healthy vegetation, there was significant difference between the heat stressed and non-stressed environments (Table 4.3). Contrast analysis revealed that under the heat stressed environment, cultivars with the dark green canopy had higher PRI and ARI, and lower NPCI values than cultivars with the bright green canopy, which suggested the pigment indices were influenced by leaf color. Normalized pigment and chlorophyll index (NPCI) was negatively correlated with chlorophyll concentrations. Cultivars with semileafless leaf and upright canopy

habit had higher WBI than cultivars with normal leaf and vining growth habit (Table 4.5). The water band index was associated with leaf water content (Penuelas et al., 1997) and a greater WBI value suggested that the upright and semileafless cultivars maintained higher leaf water content under heat stressed environments.

Table 4.3. Means of various vegetative indices of 24 pea cultivars grown across six environments, in western Canada. Means with similar letters within a column are not significantly different (P=0.05). N = 288 for environment, and N = 72 for cultivar.

Effects	NDVI	PRI	GNDVI	WBI	ARI	CRI	NPCI
Environment (E)							
R14	0.78 c	-0.006 a	0.59 c	1.09 a	3.9 cd	0.044 cd	0.300 b
R15	0.80 ab	-0.024 cd	0.6 c	1.09 a	4 c	0.062 c	0.363 a
S14	0.79 bc	-0.019 bc	0.62 ab	1.08 b	4.2 ab	0.057 ab	0.301 b
S15	0.79 bc	-0.026 d	0.61 bc	1.08 b	4 bc	0.057 bc	0.295 b
S16	0.77 d	-0.028 d	0.57 d	1.07 c	3.7 d	0.05 d	0.321 b
SN16	0.81 a	-0.013 b	0.63 a	1.09 a	4.4 a	0.066 a	0.302 b
Cultivar							
03H107P-04HO2026	0.80 ab	-0.019 d-g	0.61 bc	1.1 ab	4.2 b	0.061 ab	0.33 bc
03H267-04HO2006	0.79 a-d	-0.022 e-h	0.6 b-d	1.09 b-d	4 b-d	0.056 b-f	0.32 b-e
40-10	0.79 a-d	-0.02 d-g	0.59 de	1.08 d-j	3.8 d-f	0.051 c-f	0.329 bc
Aragorn	0.79 a-c	-0.02 d-h	0.61 bc	1.08 g-k	4.1 bc	0.054 b-f	0.295 d-f
CDC_Golden	0.77 e	-0.021 d-h	0.59 de	1.09 d-g	3.8 c-f	0.047 f	0.303 c-e
CDC_Meadow	0.79 a-e	-0.015 b-d	0.61 bc	1.09 c-f	4.1 bc	0.05 d-f	0.288 ef
CDC_Sage	0.81 ab	-0.026 g-i	0.6 b-d	1.08 d-i	4 b-d	0.067 a	0.343 b
CDC_Vienna	0.82 a	-0.011 ab	0.61 bc	1.09 d-h	4.2 b	0.057 b-f	0.314 b-e
Delta	0.80 a-c	-0.023 f-h	0.6 b-d	1.09 c-e	4 b-d	0.062 ab	0.336 b
Eclipse	0.78 b-e	-0.022 f-h	0.59 de	1.08 d-j	3.8 d-f	0.056 b-f	0.312 b-e
Kaspa	0.79 a-d	-0.019 c-f	0.59 de	1.10 a-c	3.8 c-f	0.056 b-f	0.331 bc
MFR043	0.79 a-c	-0.015 b-e	0.62 bc	1.11 a	4.2 b	0.058 a-d	0.3 c-f
Mini	0.78 b-e	-0.015 b-d	0.6 cd	1.08 h-k	3.9 b-d	0.056 b-f	0.317 **b-e**
MPG87	0.79 a-c	-0.012 a-c	0.62 b	1.08 i-k	4.2 b	0.053 b-f	0.294 ef
Naparnyk	0.78 c-e	-0.032 i	0.56 f	1.08 i-k	3.6 f	0.053 b-f	0.385 a
Rally	0.78 de	-0.011 ab	0.61 b-d	1.07 k	4.2 b	0.057 a-e	0.268 fg
Superscout	0.80 a-c	-0.006 a	0.67 a	1.09 d-g	5.4 a	0.055 b-f	0.21 h
TMP15116	0.79 a-d	-0.027 hi	0.57 ef	1.07 jk	3.6 ef	0.06 a-c	0.392 a
TMP15179	0.78de	-0.011 ab	0.6 b-d	1.08 d-j	4 b-d	0.047 ef	0.256 g
TMP15181	0.79 a-d	-0.023 f-h	0.6 cd	1.08 d-j	3.9 b-e	0.058 a-d	0.331 bc
TMP15202	0.79 a-d	-0.021 d-h	0.61 b-d	1.09 c-e	4.1 b-d	0.055 b-f	0.3 c-e
TMP15206	0.78 a-e	-0.023 f-h	0.6 b-d	1.08 d-j	3.9 b-d	0.057 a-e	0.326 b-d
TMP15213	0.79 a-e	-0.023 f-h	0.6 cd	1.08 f-k	3.9 b-e	0.05 c-f	0.314 b-e

Torsdag	0.80 a	-0.025 f-i	0.61 b-d	1.08 e-j	4.1 bc	0.067 a	0.336 b
Significance							
Environment	***	***	***	***	***	***	***
Cultivar	***	*	**	***	***	***	**
E*C	***	*	*	***	***	***	**

***, **, *Significant at P ≤ 0.001, 0.01 and 0.05, respectively. ns = non-significant at P ≤ 0.01
Note NDVI: normalized difference vegetation indices, PRI: photochemical reflectance index, WBI: water band index, CRI: carotenoid reflectance index, ARI: anthocyanin reflectance index, GNDVI: green NDVI, NPCI: normalized pigment chlorophyll ratio index

4.3.4 Phenotypic correlation between pigments, wax, vegetation indices, and canopy temperature

Leaf spectral properties are mainly influenced by pigment and wax concentrations, and therefore, most of the reflectance indices correlated with pigment and wax concentrations, and canopy temperature. Photochemical reflectance index was positively correlated with total lamina total chlorophyll (r = 0.77), carotenoid (r = 0.76) and anthocyanin (r = 0.71) concentrations. Triangular vegetation greenness was negatively correlated with total chlorophyll (r = -0.85), carotenoid (r = -0.80), and anthocyanin (r = -0.70) concentrations. Similarly, green normalized difference vegetation index (NDVI), and anthocyanin reflectance (ARI) indices had a very strong positive correlation with the above pigments (r > 0.88) for total chlorophyll, and carotenoid; and (r = 0.67) for anthocyanin). Water band index (WBI) was positively correlated (r = 0.48) with lamina wax concentration, and negatively correlated (-0.83) with canopy temperature. Anthocyanin reflectance index had strong positive correlation with all pigment traits. Carotenoid reflectance index (CRI) had significant negative correlation with carotenoid and total chlorophyll concentrations (Table 4.4).

4.3.5 Principal component analysis reveals heat tolerance traits and cultivars response to heat stress

The principal component analysis based on correlation of pigment, wax and remotely sensed traits revealed a remarkable cultivar grouping regarding traits associated with heat tolerance. The first two principal components explained 82.6% of the total variance among the cultivars. In all conditions, anthocyanin, carotenoid, total chlorophyll and vegetative indices PRI and GNDVI were positioned in close proximity with each other and distant from NPCI (Figure 4.7), which is in agreement with the negative association obtained through correlation analysis (Table 4.4). The NPCI is an indicator of chlorophyll and pigment concentrations, and increased

value of NPCI associates with less pigment absorption in the red relative to the blue region in the light spectrum. The NPCI negatively correlated with chlorophyll concentration, and its value increased when the leaf was losing chlorophyll (Table 4.4). The PCA also clearly demarcated between the normal and semileafless leaf types of the cultivars. The normal leaf cultivars were positioned towards the canopy temperature trait indicating these cultivars had greater canopy temperature than the semileafless cultivars. The PCA also revealed the significant negative association of canopy temperature with water band index and lamina wax concentration.

Table 4.4. Correlation test between lamina wax, anthocyanin, total chlorophyll, carotenoid and vegetation indices: triangular vegetation greenness (TVG), Green normalized difference vegetation index (GNDVI), Anthocyanin reflectance index (ARI), Normalized pigment and chlorophyll index (NPCI), Photochemical reflectance index (PRI), Normalized difference vegetation index (NDVI), and carotenoid reflectance index (CRI) of pea grown in field across six environments in western Canada; N = 24, averaged over four replications per environment.

Variables	Canopy temperature	Wax	Total chlorophyll	Antho-cyanin	Carote-noid	NDVI	GNDVI	PRI	NPCI	TVG	ARI	WBI
Wax	-0.72											
Total chlorophyll	-0.20	0.22										
Anthocyanin	-0.43*	0.46*	0.79***									
Carotenoid	-0.15	0.14	0.97***	0.80***								
NDVI	-0.32	-0.03	-0.02	-0.07	-0.06							
GNDVI	-0.16	0.18	0.91***	0.67**	0.88***	0.13						
PRI	-0.19	0.29	0.77***	0.71**	0.76***	-0.15	0.76***					
NPCI	-0.03	-0.23	-0.76***	-0.56**	-0.74***	0.41*	-0.81***	-0.78***				
TVG	0.21	-0.39	-0.85***	-0.70**	-0.80***	0.18	-0.90***	-0.81***	0.92***			
ARI	-0.05	0.08	0.93***	0.65**	0.89***	0.06	0.98***	0.71***	-0.79***	-0.88***		
WBI	-0.83***	0.48*	0.07	0.32	0.07	0.31	0.03	0.06	0.18	-0.05	-0.06	
CRI	-0.27	0.01	-0.50*	-0.27	-0.52*	0.63**	-0.37	-0.41*	0.72***	0.47*	-0.40*	0.34

*, **, *** indicate the correlation was significant at P < 0.05, 0.01, and 0.001 respectively.

4.4 Discussion

While heat stress limits crop productivity, heat resistant crops are equipped with one or more heat avoidance or tolerance strategies to maintain yield stability under the heat stressed conditions (Wahid et al., 2007; Hasanuzzaman et al., 2013). Protection of vital metabolic processes from excess heat and radiation load can be attained by various biochemical components, and the plant architecture including the growth habit and leaf shape (Havaux, 1989; Shepherd and Griffiths, 2006; Misra et al., 2006; Hatier and Gould, 2008). Pea has genetically diverse germplasm in regard to pigment and wax content and composition which can be involved in heat response (Havaux, 1989). The recent advancements in high throughput data acquisition technologies mostly rely on remotely sensed measurements; typically targeting canopy and leaf secondary traits including, canopy greenness and pigments, canopy temperature, plant water status, and the like. The responses of these traits to environmental stresses indicate the plants overall physiological status and stress level and their role in avoiding/tolerating the stress (Penuelas et al., 1993; Holmes and Keiller, 2002; Babar et al., 2006).

4.4.1 Chlorophyll, carotenoid and anthocyanin contribute to pea heat tolerance

Our results showed heat stress lead to a significant chlorophyll a and chlorophyll b loss, and similar results were reported on pea and other crops including maize, wheat and sorghum (Karim et al., 1999; Alexieva et al., 2001; Dutta et al., 2009; Hasanuzzaman et al., 2013; Feng et al., 2014). Such a loss in chlorophyll concentration arose either due to decreased biosynthesis or chlorophyll degradation, or a combination the both (Karim et al., 1999). Chlorophyll is a key component in light absorption and transfer, and thus chlorophyll degradation causes impaired photosynthesis and oxidative damage which consequently leads to reduced growth and yield (Berry and Bjorkman, 1980; Guo et al., 2006; Wahid et al., 2007; Hasanuzzaman et al., 2013). Dutta et al., (2009) indicated that the chlorophyll loss mostly associated with limited biosynthesis caused by enzyme malfunctioning (Nelson, 1988). For both chlorophyll a and chlorophyll b, there was a significant environment x cultivar interaction, and cultivars with a relatively stable chlorophyll content across environments including CDC Meadow, Delta, CDC Golden, Naparnyk, and TPM 15213 also had a high overall heat tolerance index (Figure 4.1).

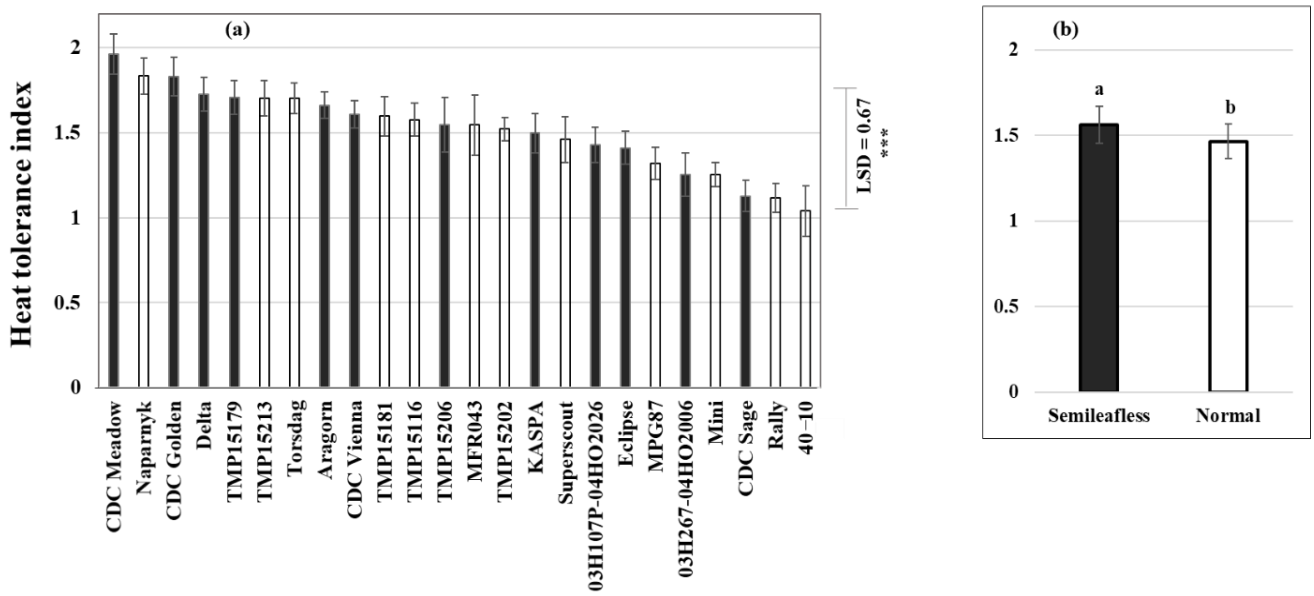


Figure 4.1. Heat tolerance index by cultivars (a), and leaf type (b) of 24 pea cultivars grown in field condition across six environments in western Canada. N = 20 for each cultivar in panel a; and N = 240 for each leaf type in panel b. Error bars are standard errors of mean.

Interestingly, I noticed an increased chlorophyll a/b ratio under the heat stressed environment (S-15), likely due to a faster chlorophyll b degradation than chlorophyll a, indicating a differential sensitivity in light-harvesting chlorophyll a/b-binding proteins complex (Plumley et al., 1995). Although, a change in chlorophyll a/b ratio associates in the plant's heat response (Cui et al., 2006; Feng et al., 2014), reports were inconsistent on how the chlorophyll a/b ratio is linked with the heat response. Some reports indicated decreased chlorophyll a/b ratio as an indicator of heat tolerance (Feng et al., 2014), while others showed the opposite (Cui et al., 2006). Chlorophyll a/b ratio had significant negative correlation (r = -60**) with heat tolerance index (Fig 4.6e) and the cultivars with relatively less (< 3.2) chlorophyll a/b ratio and high HTI included CDC Meadow, Naparynk, CDC Golden, TMP 15179 and TMP 15213, suggesting relatively low chlorophyll a/b ratio likely associates with heat tolerance (Feng et al., 2014). The chlorophyll a/b ratio shows the relative sensitivity of the light harvesting complex and the reaction center (Cui et al., 2006). Semileafless cultivars had had lower chlorophyll a/b ratio and greater heat tolerance index than cultivars with the normal leaf (Table 4.5), which agrees with the findings in chapter 3 of this thesis. While the optimal range of chlorophyll a/b ratio needs further study, both too high or too low chlorophyll a/b ratio suggests damage at the antenna complex or the reaction center respectively (Guo et al., 2006; Feng et al., 2014).

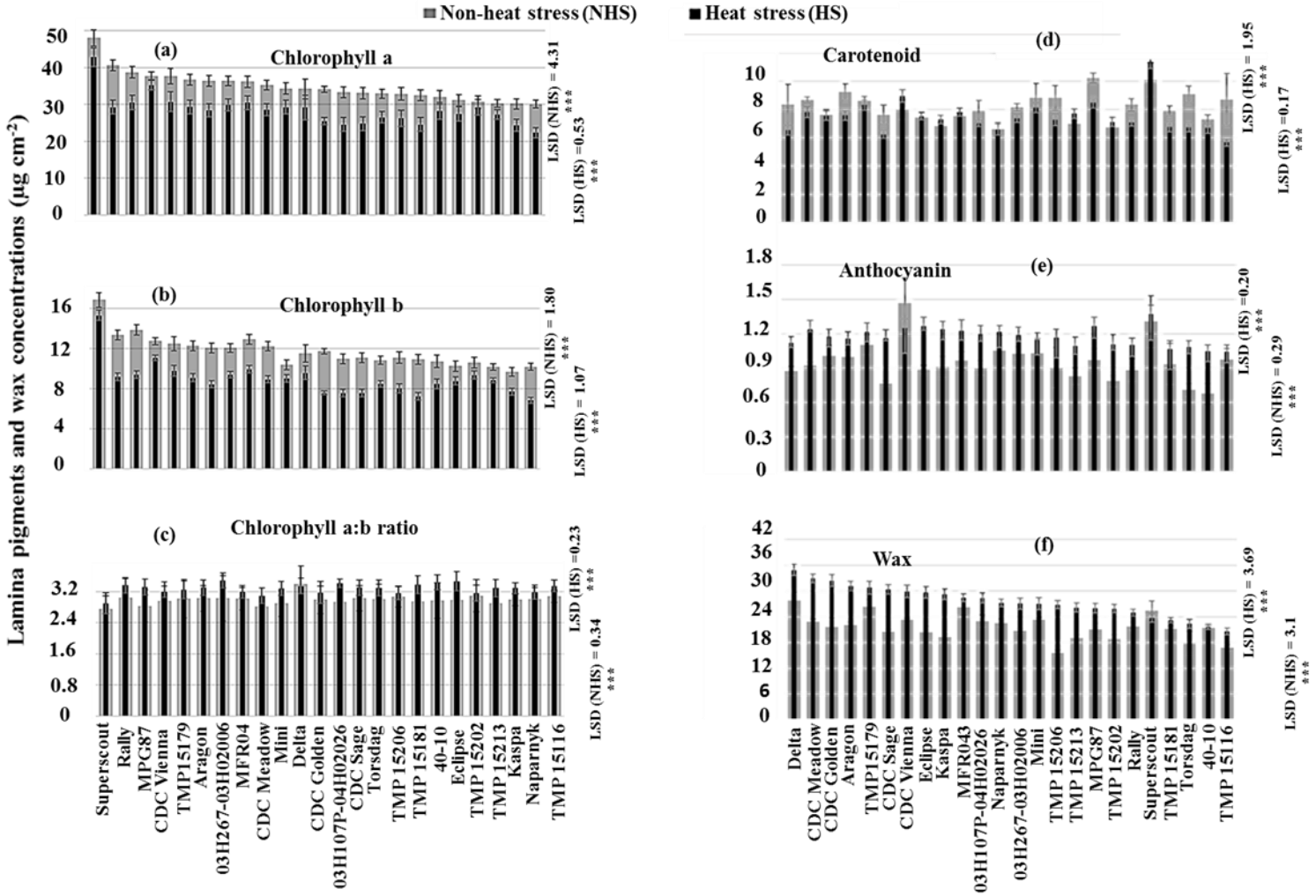


Figure 4.2. Mean lamina pigments and wax concentrations, chlorophyll a, chlorophyll b, chlorophyll a/b ratio, carotenoid, anthocyanin and wax concentrations of 24 pea cultivars grown in field under heat stressed (Late) and non-stressed (Normal) environments. Each bar represents the mean values and error bars on each bar represent standard error of mean. N = 40, for heat stressed; and N = 8, for non-stressed environments.

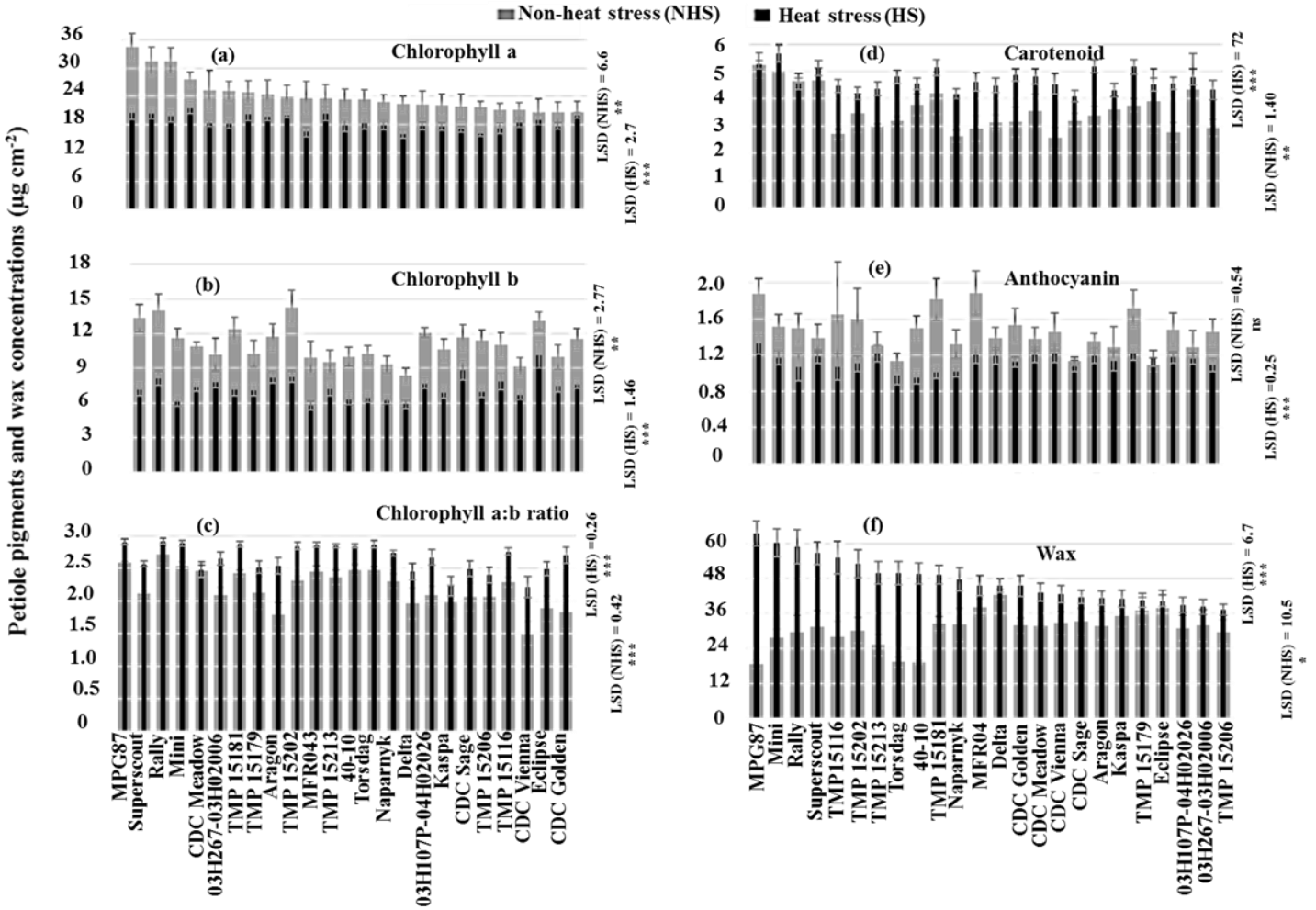


Figure 4.3. Mean petiole pigments and wax concentrations, chlorophyll a, chlorophyll b, chlorophyll a/b ratio, carotenoid, anthocyanin and wax concentrations of 24 pea cultivars grown in field under heat stressed (Late) and non-stressed (Normal) environments. Each bar represents the mean values and error bars on each bar represent standard error of mean. N = 40, for heat stressed; and N = 8, for non-heat stressed environments.

Table 4.5. Contrast analysis effects of canopy color, flower color, leaf type, plant habit and flower color on leaf lamina and petiole chlorophyll a, chlorophyll b, a/b, wax, PRI, WBI, ARI, and NPCI of pea grown in field under heat stressed and non-stressed conditions. Bright-green (N = 200 for HS & 40 for NHS), Dark-green (N = 280 for HS & 56 for NHS), Normal leaf (N = 240 for HS & 48 for NHS), Semileafless (N = 240 for HS & 48 for NHS), Upright (N = 280 for HS & 56 for NHS), Vining (N = 200 for HS & 40 for NHS), Colored (N = 120 for HS & 24 for NHS), White (N = 360 for HS & 72 for NHS).

Environ-ment	Trait	Level	HTI	Lamina pigments and wax				Petiole pigment and wax				Vegetation indices			
				chl. a	chl. b	chl. a/b	Wax	chl. a	chl. b	chl. a/b	Wax	PRI	WBI	ARI	NPCI
Heat stressed	Canopy color	dark-green	1.56*	29.9**	9.2**	3.1	28.5*	18.9	7.75*	2.68	46.9	-0.018**	1.08	4.02*	0.30*
		bright-green	1.49	26.3	7.9	3.30*	25.1	18	6.67	2.76*	46.7	-0.024	1.08	3.84	0.34
	Flower color	colored	1.42	27.8	8.5	3.33	27	18.0	7.6	2.6	43.3	-0.021	1.09	3.90	0.33*
		white	1.55*	27.9	8.7	3.29	27.2	18.7	7.2	2.7	48**	-0.020	1.08	3.96	0.30
	Leaf type	normal	1.47	27.9	8.7	3.31	24	18.8	6.7	2.83*	52.4**	-0.020	1.07	3.97	0.32
		semileafless	1.56*	27.8	8.7	3.30	30.2***	18.2	7.9*	2.49	41.3	-0.020	1.10*	3.93	0.32
	Plant habit	upright	1.57**	28.2	8.7	3.32*	29.8***	18.3	7.6*	2.57	43.3	-0.020	1.09*	3.95	0.31
		vining	1.44	27.4	8.6	3.25	23.4	18.8	6.9	2.78*	51.8**	-0.021	1.07	3.92	0.32
Non-heat stressed	Canopy color	dark-green		36.1**	12.4*	2.98	22.2*	25.0*	11.9*	2.16	30.8	-0.010*	1.08	4.49*	0.28**
		bright-green		33.1	10.9	3.00	20.2	22.5	9.9	2.27*	30.5	-0.018	1.09	4.23	0.33
	Flower color	colored		34.5	11.4	2.96	21.1	23.1	11.3	2.09	29.1	-0.009	1.09	4.23	0.29
		white		35.0	11.8	3.00	21.5	24.2*	11.0	2.25*	31.2*	-0.015	1.09	4.46*	0.31*
	Leaf type	normal		35.1	11.9	2.96	20.8	25.6**	10.5	2.42**	27.6	-0.013	1.08	4.56*	0.30
		semileafless		34.6	11.6	3.02	20.9	22.3	11.7	1.98	33.7**	-0.014	1.1*	4.23	0.30
	Plant habit	upright		34.7	11.5	3.01	22.3*	22.7	11.3	2.07	33.5**	-0.014	1.10*	4.24	0.30
		vining		35.0	12.0	2.96	20.2	25.8**	10.8	2.4*	26.7	-0.013	1.08	4.62	0.31

Note: HTC = heat tolerance index, chl. = chlorophyll, PRI = photochemical reflectance index, WBI = water band index, ARI = anthocyanin reflectance index, NPCI = normalized pigment and chlorophyll index, HS = heat stress, NHS = non-heat stress. ***, **, *Significant at $P \leq 0.001$, 0.01 and 0.05, respectively. ns = non-significant at $P \leq 0.01$.

Leaf carotenoid concentration had generally a similar trend with the total chlorophyll content. Like the chlorophyll, the carotenoid concentration decreased due to the heat stress. Carotenoid biosynthesis and accumulation was influenced by multiple factors including light and temperature stresses (Othman et al., 2014). Carotenoid are antenna pigments and have direct influence on the photosynthesis process, and the two major roles are light harvesting during photosynthesis, and minimizing photo-oxidative damage of chlorophyll molecules by dissipating excess energy in the form of heat (Havaux, 1989; Deming-Adam and Adams, 1996; Misra et al., 2006).

Contrary to chlorophyll and carotenoid, a higher concentration of anthocyanin was associated with the most heat-stressed environment. It has been reported that anthocyanin production was enhanced in response to most environmental stresses including heat, drought, and light (Shepherd and Griffiths, 2006). However, stressful environments also trigger the formation of harmful reactive oxygen species (ROS), and free radicals (Tripathy and Oelmuller, 2012). To protect plants from the harmful effects of ROS, high level of anti-oxidants is needed, and anthocyanins were reported to fulfill such a role of anti-oxidant effects in several crops (Barker et al., 1997; Hatier and Gould, 2008). Unlike the chlorophyll and carotenoid, anthocyanin concentration was higher in petiole than the leaf lamina, and the concentration showed a decreasing trend during the reproductive stage, i.e., anthocyanin concentration was higher in the early flowering stage than flower termination stages, indicating anthocyanin biosynthesis is growth stage dependent and relatively younger leaves produce higher anthocyanin (Murray and Hackett 1991). Anthocyanins protect chloroplast by reducing incident light on it and have an anti-oxidant role through scavenging reactive oxygen species (Yamasaki et al., 1996; Yamasaki, 1997).

In addition to the UV and heat protection, it has been suggested that anthocyanin accumulation under heat stress conditions associates with increased uptake and reduced transpirational water loss (Bita and Gerats, 2013; Wahid et al., 2007). My result showed lamina anthocyanin concentration had a significant negative correlation with canopy temperature (Figure 4.5c). Anthocyanins protect sensitive plant tissues by screening damaging UV radiation (Barker et al., 1997; Singh et al., 1999), and their concentration increases in response to high temperature stress (Hosseinian et al., 2008). My data showed a significant positive association between lamina wax and anthocyanin concentration, and both of them increased with the heat stress.

4.4.2 Contribution of wax as a heat tolerance trait

While roles of epicuticular wax as a drought tolerance trait was extensively reported over a range of crops (Shepherd and Griffiths, 2006; Sanchez et al., 2001; Ebercon et al., 1977; Guo et al., 2016; Willick et al., 2017), relatively little has been reported on its role as a heat stress tolerance trait. My result showed a significant variation among pea cultivars in both lamina and petiole bulk wax concentration under heat stressed, and non-stressed environments. Wax composition and concentration variability have also been reported within other pea cultivars, and between several crop species (Ebercon et al., 1977, Sanchez et al., 2001). Compared to the non-heat stressed environment, heat-stressed environment had 29.6% higher total leaf wax concentration. Moreover, during the reproductive stage wax concentration increased by 47.8 and 44.1% in heat stressed and non-stressed environments, respectively. These results indicated genetic factor (cultivar), plant age and heat stress contributed to the leaf wax biosynthesis, but the environment was the most determining factor (Shepherd and Griffiths, 2006). In addition to heat stress, drought, cold, salinity, and mechanical damage also contributed to increased wax load in several crop species (Premachandra et al., 1992; Sanchez et al., 2001; Shepherd and Griffiths, 2006; Guo et al., 2016).

In regard to heat avoidance, epicuticular wax has two major roles: protecting from excess radiation and heat by reflecting ultraviolet, visible and infrared wavelengths (Ebercon et al., 1977; Jefferson et al., 1989; Sanchez et al., 2001; Shepherd and Griffiths, 2006); and minimizing water loss through reduced stomatal and residual transpiration (Jordan et al., 1984; Premachandra et al., 1992; Sanchez et al., 2001; Guo et al., 2016; Hasanuzzaman et al., 2017). In a pilot study by adding extra wax on the leaf surfaces in field (data not shown here), I noticed that radiation reflectance both in the visible and near-infrared region was positively associated with wax concentration, a similar result was reported by Jefferson et al (1989) on *Triticeae* range grasses. Principal component analysis and correlation test clearly demonstrated negative correlation between leaf wax concentration and canopy temperature, indicating the role of wax in reflecting heat load. Previous studies on pea and other crops reported an association of epicuticular wax with improved drought tolerance (Sanchez et al., 2001; Kosma et al., 2009). As reported in chapter 6 of this thesis, drought and heat usually confounded and drought stress often leads to or aggravate heat stress.

Generally greater wax concentration was associated with decreased canopy temperature, and a higher heat tolerance index (Fig 4.5a, and Fig 4.6b). Cultivars with darker or bluish-green leaves had a higher lamina wax concentration than light green leafed cultivars (Table 4.5). Blueish,

or whitish leaf color was used in visual examinations for leaf glaucousness in wheat (Jenks et al., 1992; Shepherd and Griffiths, 2006, 2006; Willick et al., 2017). Cultivars with upright growth habits and semileafless leaves, both stress hardy traits, were associated with higher wax concentration under the stressed environment (Table 4.4). Wax accumulation positively associates with water band index, a proxy for leaf water content, indicating the leaf surface wax is minimizing water loss (Table 4.3). Generally, glaucousness or waxy leaves helps to maintain high water potential and can therefore be considered as a trait for drought tolerance (Richards et al., 1986; Ludlow and Muchow, 1990), and indirectly as a trait of heat tolerance as I noticed enough water supply moderating heat stress in a separate study. Richards et al (1986) indicated a 0.7 °C difference in leaf temperature between waxy and non-waxy wheat cultivars. I concluded that higher lamina and petiole wax concentrations minimized heat stress by protecting the plant from excess radiation and heat load. Increased wax concentration also helped to maintain leaf water content, likely due to minimized residual transpiration.

4.4.3 Spectral reflectance association with heat stress

Our stipule-level reflectance of light was measured at specific wavelengths using a spectroradiometer. The spectral reflectance in the visible (VIS) wavelengths (400–700 nm) mainly influenced by leaf chlorophyll and associated pigments such as carotenoid and anthocyanin (Jacquemoud and Ustin 2001; Holmes and Keiller 2002). Vegetation indices are proxies to estimate the content and function of various growth, pigment and water content traits (Penuelas et al., 1993; Penuelas et al., 1997; Gamon et la., 1998; Zarate-Valdez et al., 2012). Indices derived from reflectance in the visible and near infrared regions such as NDVI and its derivatives indicate vegetation greenness, photosynthesis efficiency, and rate of senescence (Babar et al., 2006; Lopez and Reynolds 2012).

Heat stress disturbs photosynthesis and leads to pigment degradation, and such effects can be indirectly traced from spectral reflectance. My data showed significant positive correlation between GNDVI and chlorophyll content (Table 4 4; Figure 4.7). Vegetation indices derived from reflectance in the near infrared region including water band index are proxies mainly for the tissue water status (Penuelas et al., 1997; Zarate-Valdez et al., 2012). My result showed a significant negative correlation between WBI and canopy temperature, and positive association between WBI and wax concentration (Figure 4.5d). Another group of VIs are those derived from the reflectance

in the visible spectral region including photochemical reflectance index, normalized pigment and vegetation index, triangular vegetation greenness, and carotenoid reflectance index. These indices are proxies for content and function, and photosynthetic efficiencies (Penuelas et al., 1993; Gamon et la., 1998). Significant positive correlation was observed between PRI and chlorophyll content, and NPCI was associated with limited pigment and high stress. Such consistent and clear trends of VIs with pigment, wax, canopy temperature and other heat stress related traits indicate the potential benefit of the indices in heat stress studies.

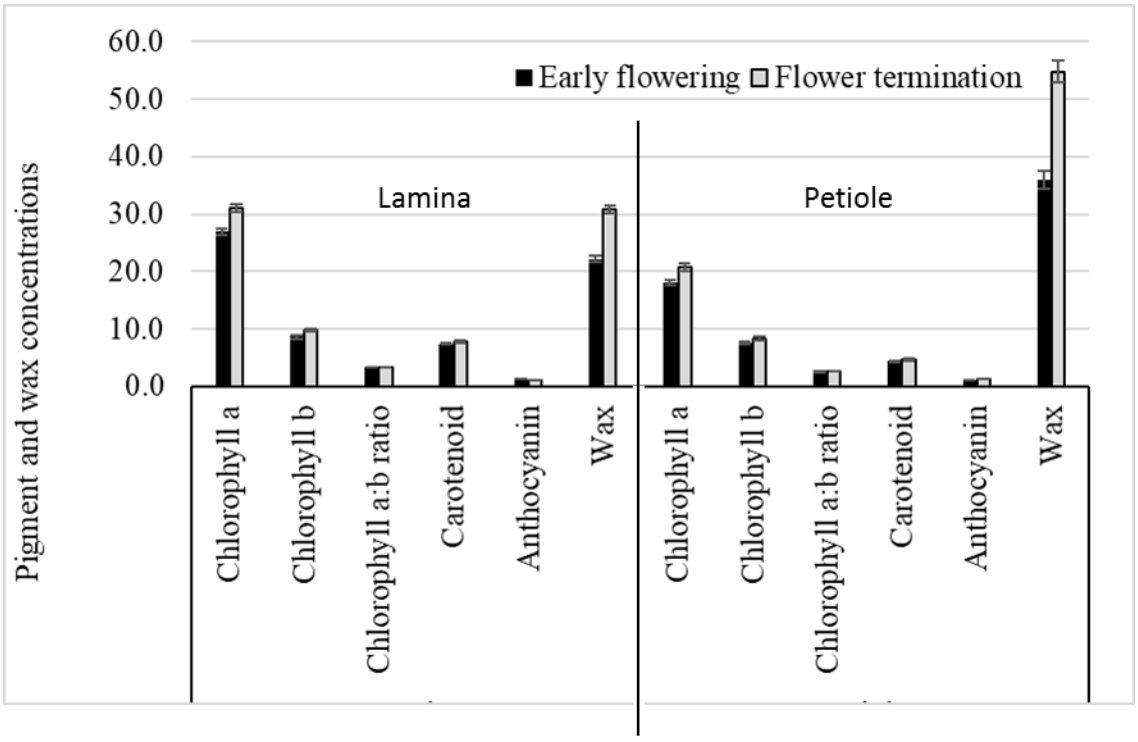


Figure 4.4. Chlorophyll a, b, a/b, carotenoid, anthocyanin and wax concentrations in leaf lamina and petiole at early flowering and flower termination stages of pea grown in field. Each bar is a mean value averaged over 24 cultivars, six environments and four replications per environment. N = 576.

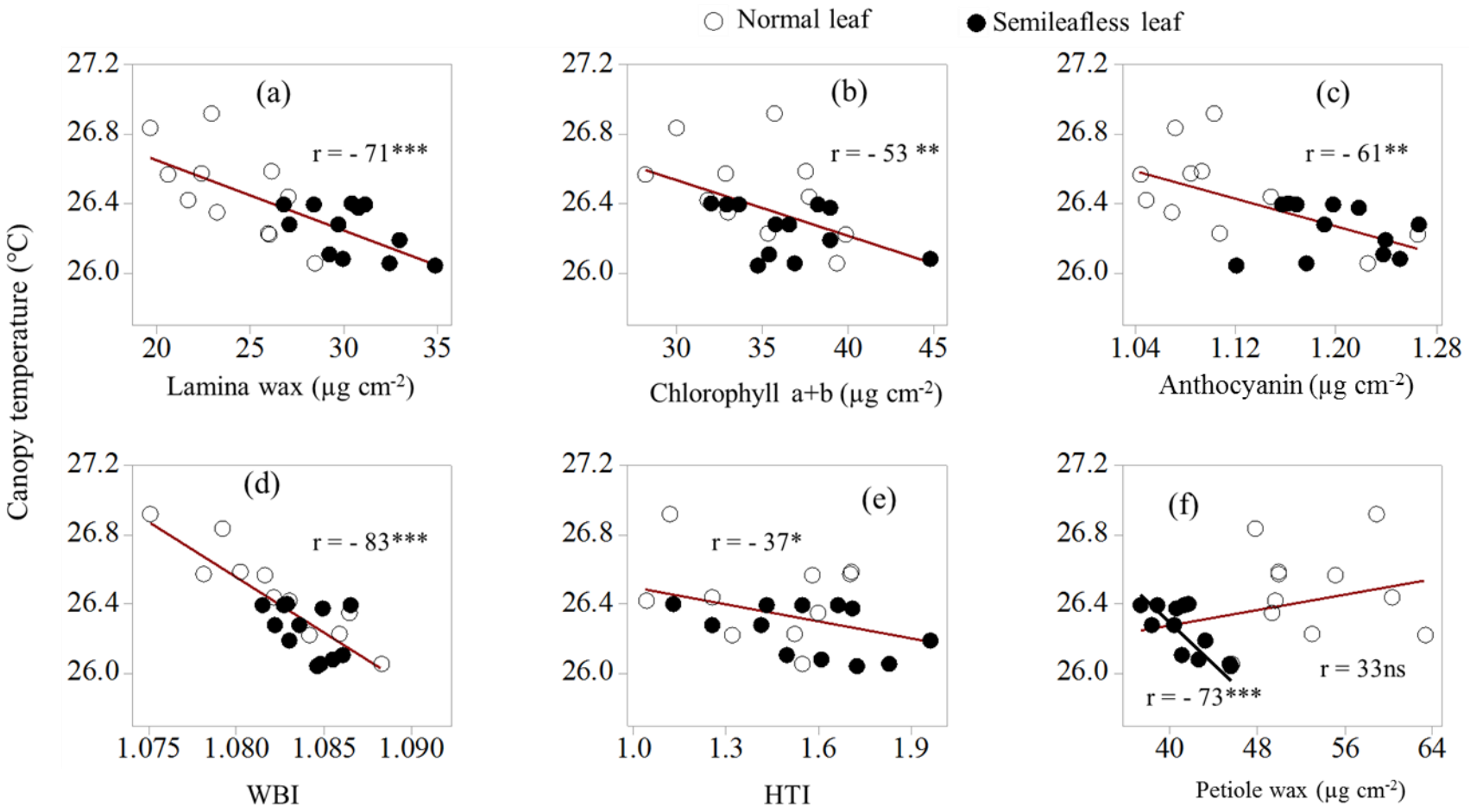


Figure 4.5. Canopy temperature relationship with pigments, wax, and vegetation indices of 24 pea cultivars (normal and semileafless leaf types) grown in field condition across six environments in western Canada. Each symbol is a cultivar averaged over six environments, and four replications per environment.

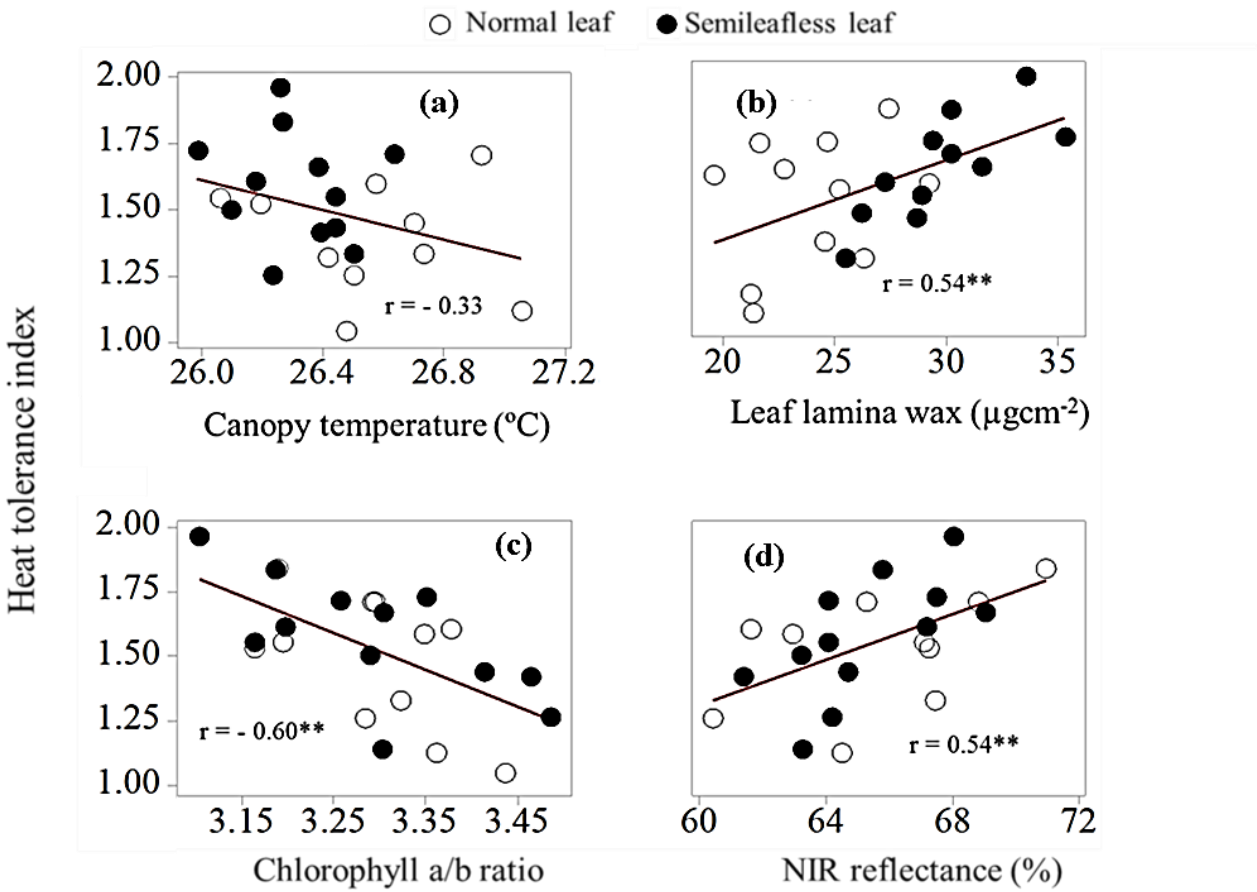


Figure 4.6. Heat tolerance index correlation with canopy temperature, lamina wax, chlorophyll a/b ratio and NIR reflectance percentage of 24 pea cultivars (normal and semileafless leaf types) grown in field condition across six environments in western Canada. Each symbol is a cultivar averaged over six environments, and four replications per environment.

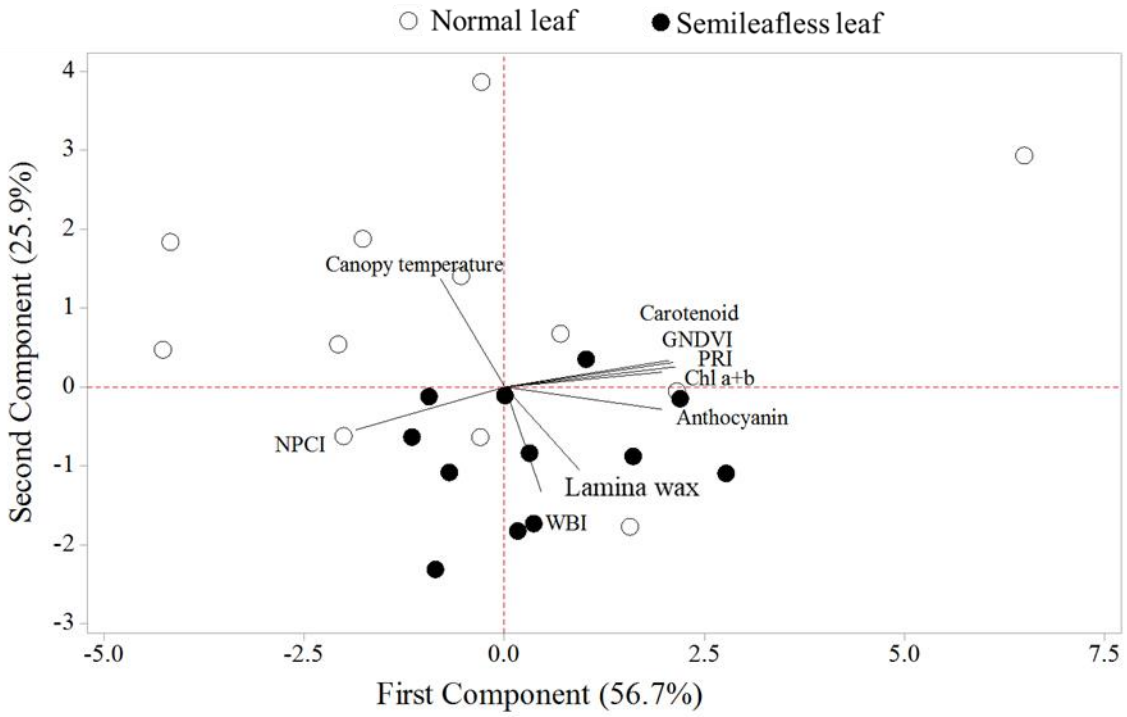


Figure 4.7. Principal component analysis of pigments, lamina wax, and vegetation indices of 24 pea cultivars grown in field across six environments in western Canada. Each is a cultivar averaged over six environments, and four replications per environment.

4.5 Conclusions

The results of this study demonstrated that heat stress reduced chlorophyll a, chlorophyll b, and carotenoid concentrations but increased wax and anthocyanin concentrations, and chlorophyll a/b ratio. Generally, leaf pigments (chlorophyll, carotenoid, and anthocyanin) both from petiole and lamina positively correlated with heat tolerance index and contributed to a lower canopy temperature. Chlorophyll a/b ratio negatively correlated with heat tolerance index. Semileafless leaf, upright canopy and bright-green leaf associates with high heat tolerance index under the heat stressed environments. Photochemical reflectance index, green normalized vegetation index, normalized pigments and chlorophyll index, and water band indices showed a consistent pattern of relationship with the pigment and wax concentrations, and with heat tolerance traits, suggesting the possibility of using the indices as indicators of the stress and their potential use in the heat stress studies.

Transition section between Chapter 4 and Chapter 5

Effects of heat stress on leaf pigments and wax concentrations and roles of these traits as a heat avoidance/trait were investigated in Chapter 4. Heat stress decreased chlorophyll a, chlorophyll b, and carotenoid but increased chlorophyll a/b ratio, anthocyanin and wax concentrations. Heat tolerance index was negatively correlated with chlorophyll a/b ratio, total chlorophyll and carotenoid concentrations. Petiole and leaf surface waxes maintained leaf water content and contributed to a cooler canopy temperature. Vegetative indices including NDVI, GNDVI, PRI, NPCI, and WBI were identified to be associated with the pigment and wax traits. The aim of the next experiment in Chapter 5 was a detailed analysis of effects of pigment and wax as heat tolerance traits and their association with leaf spectral properties by adding extra wax on leaf surfaces, and by removing the naturally existing wax and pigments.

CHAPTER 5. EXOGENOUS WAX APPLICATION, DEWAX, AND SHADE EFFECTS ON LEAF SPECTRAL PROPERTIES AND HEAT AVOIDANCE OF PEA

5.1 Introduction

In the global warming scenario, heat resistance is a desirable trait for crop yield stability. In several crops including pea, sorghum and wheat, epicuticular wax has been reported as an adaptive trait, having a protective role against high irradiation, heat load and drought (Jenks et al., 1992; Sanchez et al., 2001; Shepherd and Griffiths 2006; Willick et al., 2017). In pea, epicuticular wax conserved water by minimizing non-stomatal water loss through leaf cuticles collectively called residual transpiration (Sánchez et al., 2001). The residual transpiration, which may account for up to 28% of stomatal water loss, may occur even when the stomata are fully closed such as at night (Holmgren et al., 1965; Hasanuzzaman et al., 2017). Stay-green and high chlorophyll traits were both reported to have associations with heat and drought tolerance in several crops including wheat and sorghum (Xu et al., 2000; Cossani and Reynolds 2012). Similarly, carotenoids and anthocyanins may dissipate excess radiation and heat (Steyn et al., 2002).

Spectral reflectance from a plant's leaf or canopy at different wavelengths indicate the plant's health, vigor, and overall physiological status (Osório et al., 2012). Leaf optical properties are primarly influenced by the composition and abundance of pigments and epicuticular waxes (Jacquemoud and Ustin 2001; Holmes and Keiller 2002). Wax or pigment concentration modification by addition or removal of wax or pigments may help to better understand mechanism of heat tolerance in pea. Several researches have shown the associations between spectral indices and crop yield in a range environmental stresses (Babar et al., 2006; Xue and Su 2017).

The naturally existing waxes and leaf pigments may not attenuate all harmful ultraviolet radiation, and augmenting the leaf surface with external wax application may enhance leaf reflectance and contribute to heat avoidance under hot conditions. Such kind of studies were never done on pea and I aim to investigate roles of pigments and wax in regard to enhancing the pea heat tolerance. The specific objectives were: 1) to test effects of applied wax in the form of sprayable emulsion, 2) use wax emulsion as a canopy cooling method through enhancing canopy reflectance to reduce heat load from the sun in the UV, red edge and infrared regions, 3) investigate effects of shade on leaf pigments and how the pigments imact pea heat response. I hypothesized that exogenous application wax application on leaf surfaces as a sprayable emulsion would augment

natural waxes present on leaves and and enhance heat and radiation reflectance, and thus contributing to heat avoidance.

5.2 Materials and methods

5.2.1 Plant materials

Six pea cultivars, 'CDC Meadow', 'CDC Sage', 'Naparnyk', 'Rally', 'Superscout', 'TMP 15116' were used to investigate effects of wax and pigment manipulation on pea spectral properties and heat response. The cultivars were genetically diverse in growth habit, leaf type, leaf color and other agronomic characteristics. Supperscout and Rally, originating from the United States of America, had a dark-green leaves and high chlorophyll conentration. Naparynk and TMP15116, originating from eastern Europe, had pale-green (yellowish) leaves and low chlorophyll concentration. CDC Meadow and CDC Sage, originating from the Crop Development Center (CDC), Canada, had semileafless and upright growth habit. CDC Meadow has been one of the most widely cultivated pea cultivars in Canada and has higher heat tolernce than CDC Sage.

5.2.2 Sprayable wax emulsion preparation

The sprayable wax emulsion was synthesized by melting beeswax with distilled water and suitable emulsifiers. In order to get the desired emulsion, sodium borate (boric acid + sodium hydroxide + 125 ml distilled water), Polysorbate 20 (Tween 20), a non-ionic surfectant, were used as an emulsifying agents so as to enhance the wax-water contact. First, 600 ml distilled water was boiled, and while it was boiling 5 g of the beeswax was added, followed by 0.25 ml Borax after the wax was completely melted. The mix was kept just under boiling point and steared for five minutes until uniform emulsion was created. Then 0.1 ml of Tween 20 was added. The non-ionic surfactant Tween 20 was used to improve the surface contact of the wax with the water so the wax stayed in sprayable emulsion form and could stick to leaf surfaces. The emulsion was kept under boiling point steared for 15 minutes untill well formed wax emulsion resulted.

5.2.3 Experimental design and treatments

To each of the genotypes, four experimental treatments were applied: added wax, shade, dewax, and an untreated control. For the added wax treatment, 100 µl of the wax emulsion per stipule was applied using a pipette and then gently spread on the stipule surfaces. As the wax

concentration in the emulsion was known, the amount of wax applied to each stipule was also known (100 µg stipule^{-1}). The wax emulsion was applied on one of the fully expanded stipules at second or third node (counting from top), and each time, the second stipule of the leaf was tagged and kept as an untreated control. For each genotype, the wax emulsion was applied on 12 stipules (three stipules per replication). For the shade treatment, leaves (plant parts) were covered with aluminium foil to keep leaves dark for eight days to lower leaf chlorophyll concentration.

For the dewax treatment (removal of the naturally existing wax from the leaf surface), stipules were dipped in chloroform, and gently wiped with facial tissue paper. Spectral reflectance was measured immediately while the leaf structure was still alive and sturdy (after wax removal, leaves rapidly wilt). Untreated stipules were used as controls. The trial was laid out as a randomized complete block design with a split-plot treatment arrangement, where genotypes and leaf treatments were used as the main and sub-plot factors, respectively.

5.2.4 Plant measurements

Field level plant measurements included leaf temperature, spectral reflectance, and stomatal conductance (g_s). Stomatal conductance was measured using a portable leaf porometer (steady state Licor 1600, Li-Cor Inc, Lincoln, Nebraska, USA) on the abaxial leaf surface at solar noon. Leaf temperature was measured using an infrared (IR) fever thermometer (Equate Model 10957, Mississauga, ON) during the entire treatment duration. Spectral reflectance measurement and vegetative indices determination was exactly as described in section 4.2.5 and table 4.1 of this thesis.

Also, laboratory measurements included total wax, chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin, and carotenoid concentrations. The wax and pigments extraction and quantification were as described section 4.2.1 to 4.2.4 of this thesis.

5.2.5 Data analysis

Statistical analysis on stomatal conductance, leaf temperature, total wax, chlorophyll a, chlorophyll b, anthocyanin, carotenoid, and vegetative indices NDVI, PRI, NPCI, ARI, CRI, WBI, and on reflectance at the UV, green, red and NIR spectral regions were performed using the Mixed procedure of SAS statistical software (version 9.4; SAS Institute, Inc., Cary, NC, USA). Analysis of variance (ANOVA) with the least significant difference (LSD) test ($P < 0.05$) was used. The

effect of cultivar, wax and pigment treatments were considered as fixed effects, and replication was considered as a random effect.

5.3 Results

5.3.1 Effects of cultivars, pigment and wax treatments

Analysis of variance showed cultivar and pigment-wax manipulative treatments were significant ($P < 0.05$) for chlorophyll a, chlorophyll b, carotenoid, anthocyanin, wax, NDVI, PRI, NPCI, ARI, CRI, WBI, reflectance UV, green, red and NIR. The cultivar by manipulative treatments interaction was also significant for the chlorophyll a, chlorophyll b, carotenoid, NDVI, and reflectance at the green, red, and NIR regions (Table 5.1 and 5.2).

Compared to the untreated control, the added wax treatment increased the stipule wax concentration by 172%; however, there was 8.1% decrease in total chlorophyll concentration (Table 5.1). The anthocyanin and carotenoid concentrations, and stipule leaf temperature were not affected by the added wax treatment. Similarly, while the WBI significantly increased, pigment and biomass related vegetative indices including NDVI, PRI, ARI, and CRI were not affected by the exogenous wax application.

In contrast, the shade treatment had a very high impact on the pigment related traits including chlorophyll, carotenoid, anthocyanin, as well as on NDVI, PRI, NPCI, ARI, CRI, and on spectral reflectance percentage at the green and red spectral regions. Eight days of shade treatment reduced the total chlorophyll concentration from 53.1 to 10.3, which was over 80.6%. Similarly, the carotenoid concentration was dropped by 72% from 9.4 to 2.6. In contrast, the anthocyanin concentration was increased by 19% due to the shade but it did not affect total wax concentration, or spectral reflectance in the near infrared region. While vegetation indices NDVI, PRI and CRI respectively were decreased by 17, 166, and 33.3% due to shade treatment, the corresponding NPCI and ARI values were increased by 730 and 140% (Table 5.2). The ARI and CRI responses agree with their respective anthocyanin and carotenoid concentrations obtained from the destructive method (Table 5.1 and 5.2).

The added wax and shade treatments had unique influence on the stipule spectral property (Figure 5.4). The added wax treatment had the greatest influence of the spectral reflectance at the UV and NIR whereas the shade treatment had the greatest influence in the visible regions of the spectrum (Figure 5.4). Compared to the control, the added wax increased reflectance percentage

at the UV and NIR by 10.6 and 15.9% respectively. In the contrast, the wax removal reduced UV and NIR region reflectance by 15.2 and 12.4%, respectively. Similarly, compared to the control, the shade treatment increased the visible region reflectance by 37.0%.

The pigment and VI correlations had different patterns under different treatment conditions, and they became stronger under shade treatment compared to the added wax and control treatments (Table 5.3). Under the shade treatment, stipule total chlorophyll concentration correlated significantly with NDVI (r = 0.64), PRI (r = 0.67), NPCI (r = -0.84) and ARI (r = -0.74). Under control and added wax treatments, the above correlations were weak or non-significant. The carotenoid and VI correlations had generally similar trends to the chlorophyll and VI correlations. Interestingly, anthocyanin had a negative correlation with NDVI (r = 0.47), with PRI (r = -0.82), and with total chlorophyll (r = -0.54) under the shade treatment, whereas correlations became positive but not significant under control and added wax treatments (Table 5.3). Similarly, stipule wax concentration had an overall positive correlation with water band index (r = 0.55), and with reflectance at the ultraviolet (r = 0.84) and near infrared (r = 0.66) regions (Figure 5.3). The stipule wax concentration correlations with the spectral reflectance at the NIR region, and with the water band index, were 0.88 and 0.87, respectively. These results suggested that added wax minimized leaf water loss, evident from a significantly higher WBI.

Cultivars spectral reflectance varied under the different pigment and wax treatments. Under the added wax treatment, Superscout had the highest spectral reflectance in the NIR region followed by TMP 15116 and CDC Meadow; and Rally had the least (Figure 5.2a). Under shade treatment, Superscout and CDC Meadow had high spectral reflectance in the NIR region whereas TMP 15116 had the least (Figure 5.2a). Similarly, stipule chlorophyll and carotenoid concentration varied under the different pigment and wax treatments. Under the control treatment, Superscout had the greatest chlorophyll a, chlorophyll b and carotenoid concentration followed by Rally and CDC Meadow, and the least chlorophyll and carotenoid concentration was associated with TMP 15116. In contrast, under the shade treatment, TMP 15116 and CDC Meadow had the highest chlorophyll a and carotenoid concentrations (Figure 5.1a). Added wax and control treatments had a similar ranking in chlorophyll a, chlorophyll b and carotenoid concentrations in all cultivars, suggesting wax addition did not significantly influence pigment composition and concentration.

Table 5.1. Means of chlorophyll a, chlorophyll b, carotenoid, anthocyanin total wax concentration, and leaf temperature of six pea cultivars grown in field, and probabilities from analysis of variance (ANOVA) showing effects of treatments, cultivars, and treatment x cultivar interactions the traits. Means with a common letter within each column under each trait were not different at $P < 0.05$. N = 24 for treatment, and N = 12 for cultivar.

Effects	Chlorophyll a (µg cm^{-2})	Chlorophyll b (µg cm^{-2})	Carotenoid (µg cm^{-2})	Anthocyanin (µg cm-2)	Wax (µg cm^{-2})	Leaf temperature (^oC)
Treatments						
Control	33.7 a	11.2 a	8.9 a	1.77 b	40.2 b	28.5 b
Shade	7.1 b	2.3 c	3.9 b	2.09 a	42.1 b	28.0 b
Added wax	33.2 a	9.4 b	8.6 a	1.79 b	109.5 a	29.2 a
Cultivars						
CDC Meadow	27.5 ab	8.2 ab	7.5 abc	1.93 bc	64.8 a	28.3 a
CDC Sage	24.2 bc	8.1 ab	7.7 ab	1.54 de	66.6 a	28.2 a
Naparnyk	17.2 bc	5.5 b	5.4 c	1.73 cd	58.8 a	28.8 a
Rally	28.6 ab	7.9 ab	7.2 abc	2.2 b	67.3 a	28.5 a
Superscout	36.7 a	10.6 a	9.3 a	2.55 a	68.6 a	29.0 a
TMP 15116	15.8 c	5.4 c	5.8 bc	1.35 e	57.5 a	28.8 a
Significances						
Cultivar (c)	***	***	***	***	ns	ns
Treatment (t)	***	***	***	*	***	***
c*t	***	***	**	ns	ns	ns

***, **, *Significant at $P \leq 0.001$, 0.01 and 0.05, respectively. ns = non-significant at $P \leq 0.01$.

Table 5.2. Means of various vegetation indices, normalized difference vegetation index (NDVI), photochemical reflectance index (PRI), normalized pigment and chlorophyll ratio index (NPCI), anthocyanin reflectance index (ARI), carotenoid reflectance index (CRI), water band index (WBI), and reflectance in ultraviolet (UV), green, red, and near infrared (NIR) spectral regions of six pea cultivars grown in field, and probabilities from analysis of variance (ANOVA) showing effects of treatment (control, shaded, added wax and dewax treatments), cultivars, and treatment x cultivar interactions the traits. Means with a common letter within each column under each trait were not different at $P < 0.05$. N = 16 for cultivar, and N = 24 for treatment.

Effects	NDVI	PRI	NPCI	ARI	CRI	WBI	NIR	RED	GREEN	UV
Treatment										
Control	0.77a	-0.03b	0.03c	-0.5c	0.06a	1.06b	56.6b	7.7b	15.3b	7.2ab
Shade	0.67b	-0.08c	0.25a	0.2b	0.04b	1.05b	55.9b	12.0a	23.5a	6.7abc
Added wax	0.78a	-0.04b	-0.008c	-0.51c	0.06a	1.08a	65.8a	8.5b	16.5b	10.1a
Dewax	0.74a	0.004a	0.09b	1.24a	0.05a	1.05b	52.2c	6.4c	12.1c	6.0c
Cultivar										
CDC Meadow	0.74ab	-0.029a	0.07b	0.6a	0.06a	1.09a	60.8a	8.7ab	16.7bc	6.4abc
CDC Sage	0.74ab	-0.039ab	0.09b	0.24ab	0.05a	1.07ab	57.1b	7.3b	14.9c	5.6c
Naparnyk	0.72b	-0.065b	0.17a	0.15ab	0.05a	1.07bc	54.7ab	9.4a	20.1a	6.6ab
Rally	0.72b	-0.051ab	0.07b	0.12ab	0.06a	1.03cd	51.9b	7.9 ab	15.6bc	5.8bc
Superscout	0.78a	-0.016a	0.07b	-0.22b	0.06a	1.06b-d	60.4a	7.1 b	12.0d	6.2bc
TMP 15116	0.70b	-0.032ab	0.08b	-0.22b	0.05a	1.04d	56.5ab	9.2a	17.9ab	7.4a
Significance										
Cultivar	***	**	***	***	ns	***	*	*	***	*
Treatment	***	***	***	***	**	**	***	***	***	***
CxT	***	ns	*	ns	ns	ns	ns	*	*	ns

***, **, *Significant at $P \leq 0.001$, 0.01 and 0.05, respectively. ns = non-significant at $P \leq 0.01$.

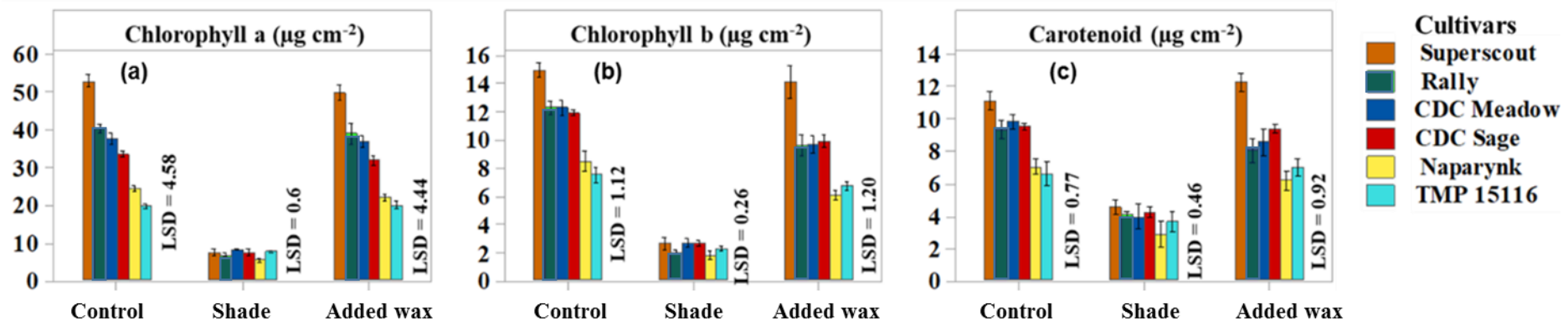


Figure 5.1. Chlorophyll a (a), chlorophyll b (b), and carotenoid concentrations of pea stipule under control, shade and added wax treatments in field. Each bar represents the mean values and error bars on each bar represent standard error of mean (N = 4). The respective least significant difference (LSD) values for each trait and treatment is shown in the figure.

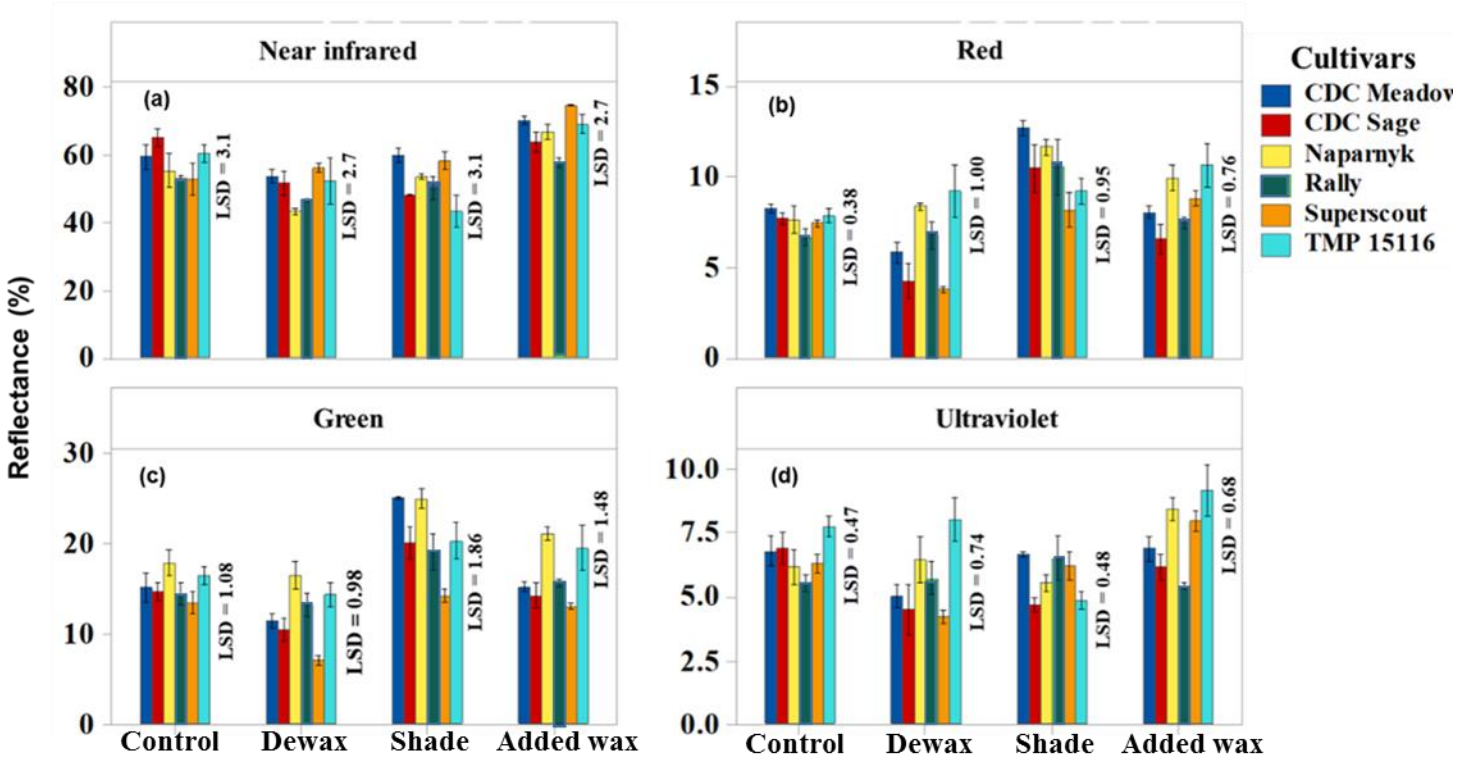


Figure 5.2. Spectral reflectance percentage in near infrared (a), red (b), green (c), and ultraviolet (d) regions of pea stipule in grown under the control, shade, and added wax treatments in field. Each bar represents the mean values and error bars on each bar represent standard error of mean (N = 4). The respective least significant difference (LSD) values for each trait and treatment is shown in the figure.

5.4 Discussion

5.4.1 Wax treatment effects on pea heat avoidance

To cope with the environmental stresses including heat and drought, plants have developed various physiological and biochemical modifications as an avoidance or tolerance mechanisms (Hasanuzzaman et al., 2013). Radiation and heat reflectance has been reported as a heat avoidance strategy during hot days (Peñuelas et al., 2004; Cossani and Reynolds 2012), which was reportedly associated with leaf surface properties such as wax and pigment composition and concentrations (Shepherd and Griffiths 2006). My results demonstrated the enhancing of pea heat avoidance by exogenous wax application. Wax application increased spectral reflectance both in the UV and NIR spectral regions in all cultivars. My results are in agreement with Holmes and Keiller (2002) and Grant et al (1995) which reported a positive association between epicuticular wax concentration and reflectance percentage both in the UV and NIR regions.

The greater spectral reflectance in the UV region protects the leaf from the high energy UV damage (Holmes and Keiller 2002). Similarly, the greater reflectance in the NIR regions decreases heat load and associates with vigor and overall plant health (Babar et al., 2006). Epicuticular waxes enhance leaf radiation reflectance and thus protect leaves from excess radiation and heat associated damage. Similar result was reported in pea and sorghum (Grant et al., 1995; Gonzalez et al.1996; Shepherd and Griffiths 2006). In contrast, wax removal (dewax), lead to decreased reflectance in the UV and NIR regions, suggesting loss of a protection from heat and radiation damage (Holmes and Keiller 2002). The exogenous wax application slightly increased leaf temperature, but significantly decreased stomatal conductance (Table 5.1 and Figure 5.5). Such reduction in stomatal conductance has been reported as a strategy to minimize water loss (Sánchez et al., 2001). My result further demonstrated, the wax application was associated with a higher water band index, indicating higher leaf water content (Figure 5.3c).

Protection against excess radiation through the reflection of visible and infrared wavelengths (Jefferson 1994; Shepherd and Griffiths 2006), and minimizing water loss through reduced residual transpiration are among the most reported functions of wax (Jordan et al., 1984; Grant et al., 1995; Gonzalez et al.1996; Sánchez et al., 2001; Shepherd and Gritt, 2006). My data demonstrated a strong positive correlation between wax concentration and reflectance percentage both at the UV and NIR regions which suggests the role of wax as a heat avoidance trait by avoiding heat load. Generally, a leaf or canopy's spectral reflectance is strongly dependent on leaf

surface topographic features including wax, leaf hairs, and pigment composition (Holmes and Keiller 2002). In wheat, heat tolerant cultivars were associated with high wax concentration (Cossani and Reynolds 2012). Similarly, an increased wax concentration enhanced drought tolerance in pea mainly by minimizing water loss through residual transpiration (Sánchez et al., 2001). Though the exogenous wax application increased leaf temperature, the naturally existing wax concentration was inversely associated with canopy temperature (data not shown here). Sanchez et al (2001) indicated epicuticular wax as a mechanism to decrease residual transpiration water loss in pea. Generally, glaucousness or waxy leaves helps to maintain high water potential and can therefore be considered as a trait for drought tolerance (Jenks et al., 2002), and indirectly as a trait of heat tolerance because I noticed a sufficient water supply could moderate heat stress in a separate study.

5.4.2 Pigment effects on vegetation indices and pea heat avoidance

Shading reduced chlorophyll a, chlorophyll b and carotenoid, but increased the anthocyanin concentration (Table 5.1). Effect of shade on anthocyanin biosynthesis varies and a study conducted on *Gynura bicolor* reported an increased anthocyanin by shading (Fukuoka et al., 2014) whereas Steyn et al (2002) emphasized the need of light for anthocyanin biosynthesis. Although there was a slight increase in the UV reflectance due to the shade treatment, the major shading impact on the leaf spectral reflectance was observed in the visible (VIS) wavelengths (400–700 nm) of the spectrum, and the influence in the near infrared region was not significantly different from the control. The loss of chlorophyll from shading was associated with less light absorption in the red regions and limited photosynthesis efficiency (Gamon et al., 1997) as explained in PRI value of less than -0.05 (Table 5.2). Vegetative indices (VIs) determined from the spectral reflectance indicate the overall physiological status and the plants stress tolerance level (Gamon et al., 1997; Xue and Su 2017). Generally, three main categories of traits can be estimated from the different VIs based on the reflectance wavelengths (Xue and Su 2017). The first group VIs includes those indices derived from the visible spectral region, PRI, NPCI, and CRI (Gamon et al., 1997; Peñuelas et al., 2004). Shading had the most obvious impact on the visible spectral region. The VIs determined from the shade treatment significantly varied from the added wax and untreated control. Photochemical reflectance index is a direct indicator of the plant's photosynthetic radiation use efficiency (Gamon et al., 1997; Babar et al., 2006; Porcar-Castell et

al., 2012). Although PRI value varies depending on several factors such as plant species, the degree of stress, nutrient availability, light intensity and others, higher value (close to zero) generally indicates a 'healthy' plant status (Gamon et al., 1997).

Table 5.3. Pearson correlation coefficients of pigments (total chlorophyll, carotenoid, and anthocyanin), and various vegetation indices under shade (bottom left), and control and added wax treatments (upper right with italic fonts). Correlation coefficients with bold indicate significant correlations ($P < 0.05$). N = 24 for shade, and N = 48 added wax and control treatments.

	NDVI	PRI	NPCI	WBI	ARI	Total chlorophyll	Carot-enoid	Wax	Antho-cyanin
NDVI		***0.42***	*-0.08*	*0.21*	*0.13*	*0.26*	*0.22*	*-0.04*	*0.24*
PRI	**0.55**		*0.24*	*0.14*	***0.37***	***0.37***	*0.33*	*-0.35*	***0.40***
NPCI	**-0.74**	**-0.77**		***-0.41***	*0.30*	*0.00*	*-0.22*	***-0.59***	*0.11*
WBI	0.05	0.19	-0.23		*0.08*	*-0.13*	*0.17*	*0.22*	*-0.15*
ARI	**-0.58**	**-0.77**	**0.74**	-0.03		*0.01*	*-0.28*	*0.19*	*0.17*
Total chlorophyll	**0.64**	**0.67**	**-0.84**	0.27	**-0.74**		***0.66***	*-0.12*	***0.73***
Carotenoid	**0.49**	**0.60**	**-0.79**	0.22	**-0.55**	**0.84**		*-0.12*	***0.42***
Wax	0.21	0.24	**-0.45**	0.31	-0.21	0.39	0.32		*-0.08*
Anthocyanin	**-0.47**	**-0.82**	**0.62**	-0.12	**0.67**	**-0.54**	-0.45	-0.15	

NDVI: normalized difference vegetation index, PRI: photochemical reflectance index, NPCI: normalized pigment and chlorophyll index, ARI: anthocyanin reflectance index.

My results also showed a significant correlation between the PRI and chlorophyll concentrations. Researches also showed significant association between PRI and net CO_2 uptake and radiation use efficiency (Gamon et al., 1997). Photochemical reflectance index was reported to associate with xanthophyll cycle pigments and the association varies depending the degree of environmental stresses including excess radiation and heat (Gamon et al., 1997; Demmig-Adams 2005). The xanthophyll protects plants from oxidative stress resulting from excess radiation, drought, heat and other stresses (Latowski et al., 2011). In dissipating excess energy, the xanthophyll cycle responds rapidly by de-poxidation of violaxanthin into zeaxanthin (Demmig-Adams and Adams, 1992; Filella et al., 2009). Photochemical reflectance index strongly

correlates with the de-epoxidation state of xanthophyll pigments (Demmig-Adams and Adams, 1992; Gamon et al., 1997).

The second group involves reflectance in the visible and near infrared regions from which VIs that indicate vegetation vigor, greenness, and rate of senescence (Babar et al., 2006). The most common VIs of this group includes NDVI and its derivatives. The shade treatment that led to chlorophyll loss, significantly reduced NDVI in all pea cultivars. In chapter 4, I reported that heat stress leads to chlorophyll degradation in pea, and several studies reported that stay green under environmental stress is a trait directly associates with stress tolerance (Cossani and Reynolds 2012). The NDVI along with other VIs can thus indicate the crop's stress tolerance level.

The third group involves vegetation indices derived from the near infrared region reflectance, which are proxies mainly for the tissue water status (Penuelas et al., 1997; Zarate-Valdez et al., 2012). The typical index in this group is water band index band index (WBI) (Penuelas et al., 1997). My result showed a significant negative correlation between WBI and leaf temperature, and positive association between WBI and wax concentration (Figure 5.3).

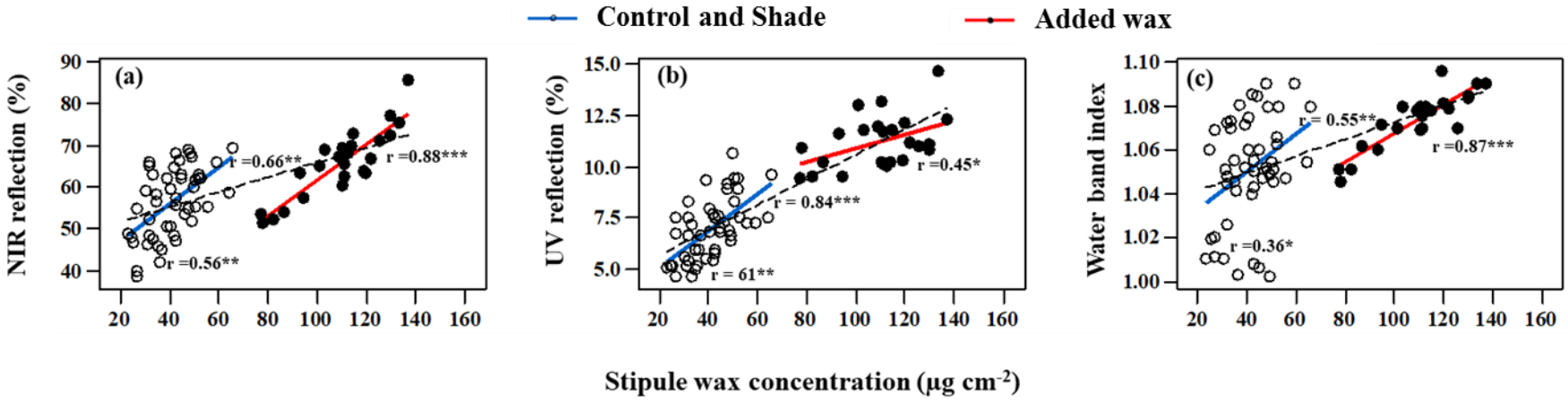


Figure 5.3. Pea stipule wax concentration correlation with reflectance percentage in near infrared (NIR, a), ultraviolet (UV, b), and water band index (c). The full black circles in each panel represented the added wax treatment, and the white empty circles in each panel represent the control and shade treatments. The three correlation coefficients (r) in each panel are for the added wax treatments (in the upper right corner), overall (in the middle) and shade and control (lower left corner). The blue, dash and red lines are fitting lines for control (shade), overall, and added wax treatments, respectively.

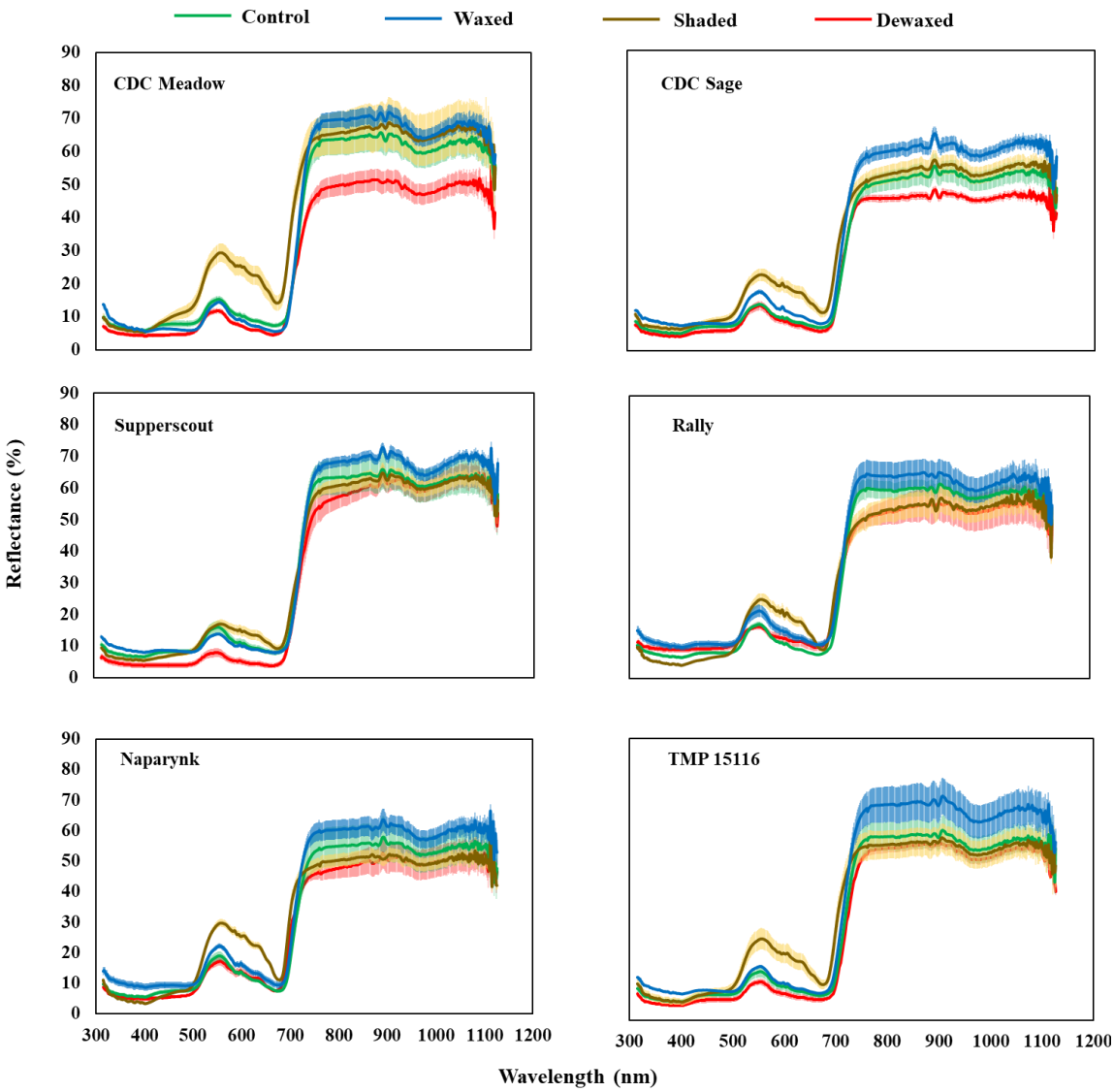


Figure 5.4. Six pea cultivars spectral reflectance percentage from 315-1120 nm wavelength under control, added wax, dewax, and shade treatments, averaged over four replications per treatment at each wavelength. Error bars at each wavelength are standard error of mean (n = 4).

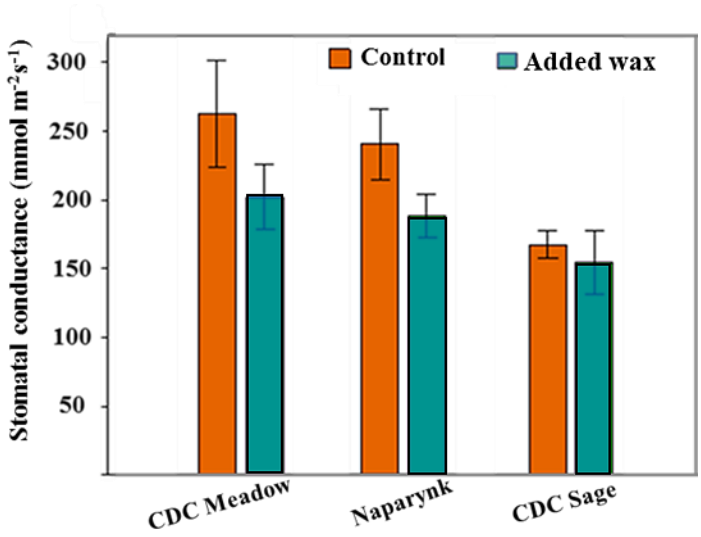


Figure 5.5. Stomatal conductance of three pea cultivars grown in field under added wax and control condition. Error bars on each bar are standard error of mean (n = 12)

5.5 Conclusions

Leaf surface wax played two roles as a heat avoidance trait: 1) reducing radiation and heat load by enhanced reflectance both in the high energy ultraviolet radiation and in the near infrared regions, and 2) minimizing water loss due to the decreased stomatal conductance and higher water band index with exogenous wax treatment. The possibility of exogenous wax application to leaf surfaces to augment the naturally existing wax content and enhance the plant's heat avoidance capacity is a novel finding. On the other hand, the shading treatment led to loss of chlorophyll and carotenoid content and function. The overall physiological and biochemical status of a plant can be studied from spectral measurement without involving costly and time consuming laboratory procedures.

Transition section between Chapter 5 and Chapter 6

Exogenous wax application, dewax, and shade effects on leaf spectral properties and heat avoidance were elucidated in Chapter 5. The added wax treatment contributed to more radiation reflections in the ultraviolet and near infrared regions of the light spectrum. The enhanced radiation reflection in the UV and NIR regions associates with heat avoidance. The added wax treatment minimized water leaf water loss. The dewax treatment had the lowest UV and NIR radiation reflection in all cultivars. The chlorophyll loss due to shading had the highest reflectance in the visible spectral regions demonstrating limited light absorption and thus photosynthesis efficiency. I demonstrated the possibility of exogenous wax application on leaf surfaces to augment the naturally existing wax content and enhance the plants' heat avoidance capacity. Heat stress usually confounds with water deficiency stress and the aim of the next experiment in Chapter 6 was to examine gas exchange, growth and seed yield response of pea under individual or combined occurrence of heat and drought, and to examine the response pattern and tolerance of four cultivars against individual or combined stresses of drought and heat.

CHAPTER 6. INDIVIDUAL AND COMBINED EFFECTS OF HEAT AND DROUGHT ON GAS EXCHANGE, GROWTH AND SEED YIELD OF FIELD PEA

6.1 Introduction

Concurrent occurrence of drought and heat stresses is a very typical situation in semi-arid areas such as the western Canadian Province of Saskatchewan, where the average annual and seasonal temperature has been increasing over the last decades (Cutforth and Judiesch 2012), and the daily maximum temperature during summer may reach or exceed 35 ºC for several days (Bueckert et al., 2015). Moreover, the trend of annual precipitation had an erratic nature with a significant reduction in recent years (Mekis and Vincent 2011; Rayne and Forest 2012). Such an increase of temperature coupled with moisture stress negatively impacted crop production (Kutcher et al., 2010; Bueckert et al., 2015; Lesk et al., 2012). Understanding of the individual or interactive effects of drought and heat, and plants' response pattern is essential to improve crops tolerance to multiple environmental stresses (Mittler 2006)

Presence of multiple environmental stresses can alter the morphological, phenological and physiological responses of crops (Wahid et al., 2007). Under such conditions, crop productivity mostly relies on the ability of crops to escape, resist or tolerate multiple stresses (Prasad et al., 2011). Combined occurrence of drought and heat decrease the rate of carbon assimilation and affect source-sink relationship, and thereby net photosynthesis (Guilioni et al., 2003; Prasad et al., 2008). Though drought and heat usually occur concurrently, many prior studies have been conducted on either drought or heat at a time but not both together (Martin et al., 1996; Beebe et al., 2013; Jiang et al., 2015). The combined occurrence of drought and heat is a typical example for the simultaneous occurrence of multiple stress factors and they are mostly inter-related (Sadras et al., 2013). High temperature is often accompanied with low water supply, and drought usually leads to heat stress. Crops generally have unique responses to individual or combined stresses, and their response to combined stress cannot be directly extrapolated from the responses of stress factors applied independently (Mittler 2006; Prasad et al., 2011). Some reports have indicated that combined drought and heat could result in different forms of interactions that would lead to additive, synergistic or antagonistic outcomes (Nicolas et al., 1984; Mittler 2006; Prasad et al., 2011)

Pea is a sensitive crop to heat and drought, factors which contribute to a significant yield loss (Guilioni et al., 2003; Sadras et al., 2012; Bueckert et al., 2015). However, there is insufficient evidence as to what degree the interactive effects of drought and heat would affect the morphological and physiological responses of pea. Understanding of the interactive effects of combined stresses and plants response to individual and combined stresses would lead to the improvement crop tolerance to multiple environmental stresses. To maintain or improve yield performance in a drier and hotter climate, new cultivars need to be resistant to individual and the combined effects of heat and drought. The specific objectives of this study were 1) to examine gas exchange, growth and seed yield response of pea under individual or combined occurrence of heat and drought, and 2) to examine the response pattern and tolerance of four cultivars against individual or combined stresses of drought and heat. I hypothesize drought and heat have both unique and common impacts on pea, and sufficient water supply moderates the heat stress effects due to enhanced transpiration cooling.

6.2 Materials and Methods

6.2.1 Plant materials

Four pea cultivars: CDC Golden, CDC Meadow, CDC Sage, and Cooper, were used to investigate individual and combined effects of drought and heat on gas exchange, growth and yield of pea. CDC Golden and CDC Meadow are widely grown cultivars under Saskatchewan conditions and perhaps they have better heat stress tolerance whereas CDC Sage and Cooper were relatively sensitive to heat stress.

6.2.2 Treatment combinations and growth conditions

The study was conducted in controlled growth chambers, where the duration and intensity of heat and water treatments were controlled. The experiment had four levels of stress and four cultivars. The four stress treatments were the non-stressed control (no water deficient), heat stress but with optimal water supply referred to as ‘heat’, water-deficient stress under optimal temperature condition referred to as ‘drought’, and the combined stresses of heat and drought referred to as ‘combined’. Before starting the stress regimes, all pots were maintained at 70% of saturated weight to avoid drainage and excess water in the root zone of the pots. The drought stress was initiated by limiting watering to 40% evapotranspiration (ET) for 12 days, started three days

before the onset of heat stress. The control and heat regimes were watered 100% ET, and the drought and combined stress regimes were watered to 40% ET. Plants under all treatment condition were watered every day. The heat treatment started right after the appearance of flower on second reproductive node, and continued for seven days.

The experiment was laid out as a completely randomized design with a factorial treatment arrangement with four replications. The experiment was repeated with a similar treatment structure but different randomization. A total of sixty-four pots of 7.6 L volume were seeded three seeds per pot with Sunshine Gro ® 138 mixes (Seba Beach, AB, Canada) and about 20 g pot^{-1} slow-release fertilizer (14-14-14, Type 100, Nutricote ® 140, Brampton, ON, Canada). Plants were thinned to two seedlings per pot about one week after emergence. To avoid any stress associated with nutrient deficiency, plants were supplied with a half strength modified Hoagland's nutrient solution in seven stocks as, KNO_3, Ca $(NO_3)_2 \cdot 4H_2O$, Iron EDTA, $MgSO_4 \cdot 7H_2O$, NH_4NO_3, KH_2PO_4 and micro-nutrients (H_3BO_3, $CuSO_4$, $ZnSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $Na_2MoO_4 \cdot 2H_2O$) once in a week starting from two weeks after emergence and stopped after pod set. Plants were watered daily as per their specified treatment.

CDC Golden and CDC Meadow were seeded 7 days after CDC Sage and Cooper so that the four cultivars would flower at similar dates. All plants were grown at 24/18 °C day/night temperature with a 16 hours photoperiod in every 24 h cycle at an irradiance of 450-500 µmol photons m^{-2} s^{-1}. Plants for the heat stress treatment were then transferred to a heat stressed chamber (35/18 °C day/night temperature) for seven days. Conditions in the heat stressed growth chamber were 16 h light with temperature increasing from 18 °C by 3 °C increments to 35 °C over 5 hours, maintained at 35 °C for 6 hours, and decreased over 5 hours to 18 °C in the night, during the treatment duration. The temperature of the growth chamber was raised by 10 °C (from 25-35°C) during the seven days of heat and combined treatments.

6.2.3 Measurements

Stomatal conductance (g_s) was measured on a fully expanded uppermost stipule using a portable leaf porometer (steady state Licor 1600, Li-Cor Inc, Lincoln, Nebraska, USA) on the abaxial leaf surface every day at noon prior to watering plants. Daily ET measurement was taken gravimetrically based on pot weight difference: ET = Pot weight after irrigation–Next day pot weight just before irrigation. Chlorophyll concentration was estimated on fully expanded stipules

using a hand held SPAD 502 meter. The SPAD measurements were taken roughly twice a week before, during and after the treatment duration, and each time two leaves per pot were measured and the average value was taken as a data point. Leaf temperature was measured on a fully expanded upper most stipule every day at noon prior to watering using an infrared (IR) fever thermometer (Equate Model 10957, Mississauga, ON) for the entire treatment duration. The number of reproductive nodes per plant was determined by counting the number of reproductive nodes on the main stem from the first flowering node to the last flowering node at maturity. The number of main stem pods per plant (pods) was determined by counting the total number of pods on the main stem that have contained at least one viable seed. Pod set ratio was calculated as the number of pods on the main stem divided by the number of main-stem reproductive nodes. Stem diameter was determined using a calibrated electronic digital caliper (Empire Level 2789, China) between the second and third node. The digital caliper had a precision of 0.01mm. Plant height and reproductive main-stem length was measured at physiological maturity.

6.2.4 Data analysis

Statistical analysis on each variable was performed using the mixed procedure of SAS statistical software (version 9.4; SAS Institute, Inc., Cary, NC, USA) after checking for normal distribution of residuals and homogeneity of variance. Analysis of variance (ANOVA) was first performed and when significant, means were separated by the least significant difference (LSD) test ($P < 0.05$). The effect of stress treatments, cultivar and their interaction were considered as fixed effects, and replication was considered as random effects.

6.3 Results

6.3.1 Stomatal conductance

Stomatal conductance (g_s) was significantly affected by stress treatments and cultivars, but the cultivar x treatment interaction was not significant (Table 6.1). Compared with the control, stomatal conductance was reduced by 3.5, 48.2 and 49.2% respectively due to heat, drought and combined stresses (Table 6.2). Drought and combined stresses had similar effects on stomatal conductance. Cultivars responded differently to the stress treatments; CDC Meadow and CDC Golden had greater conductance than CDC Sage and Cooper (Table 6.2). Compared to the control, heat and the combined stresses increased the conductance during the first three days then decreased afterwards (Figure 6.3d). Heat stress triggered stomatal opening for transpiration cooling, but drought stress induced stomatal closure to minimize transpirational water loss.

Table 6.1. Significance of cultivars (C), environment (E) and C*E interaction on various physiological, growth and yield traits of pea. Environment had four levels (Drought, Heat, Combined and Control), and the pea cultivars were CDC Golden, CDC Meadow, CDC Sage and Cooper.

Measured variables	Cultivar	Stress regime	Cultivar x stress regime interaction
Stomatal conductance	**	***	ns
Cumulative evapotranspiration	***	***	***
Leaf temperature	***	***	***
Chlorophyll content (SPAD)	*	***	ns
Duration of flowering	***	***	ns
Stem diameter	***	***	ns
Plant height	***	***	ns
Reproductive stem length	**	***	*
Number of total nodes	*	***	ns
Number of reproductive nodes	***	***	**
Number of pods per plant	***	***	***
Pods to nod ratio	***	***	***
Single grain weight	***	***	ns
Grain number	***	***	**
Yield	***	***	***

***, **, *Significant at $P \leq 0.001$, 0.01 and 0.05, respectively. ns = non-significant at $P \leq 0.01$

Table 6.2. Main effects of environment and cultivar on physiological and yield performance of four pea cultivars (Cooper, CDC Golden, CDC Meadow, and CDC Sage) grown under four environments (Control, Heat, Drought and Combined) in controlled plant growth chambers. Data are the mean of levels of environments and cultivars. Means with a common letter within each column under each trait were not different at $P < 0.05$. N = 16 for stress regimes, and N = 16 for cultivars.

Effects	**Stomatal conductance ($mmolm^{-2}s^{-1}$)**	**Cumulative evapotranspiration (mm)**	**SPAD**	**Leaf temperature (^oC)**	**Pod numbers**	**Seed size (g)**	**Seed numbers**	**Seed yield ($g\ plant^{-1}$)**
Stress regimes								
Control	313 a	4.61 a	48.6 a	24.1 d	10.1 a	0.23 a	58.1 a	13.5 a
Heat	302 a	4.60 a	45.4 b	33.1 b	7.6 b	0.22 b	43.9 b	9.0 b
Drought	162 b	2.80 b	42.5 c	25.2 c	6.3 c	0.18 c	36.8 b	5.9 c
Combined	159 b	2.72 b	40.9 d	35.3 a	4.3 d	0.18 c	18.6 c	3.9 d
Cultivars								
Cooper	225 b	3.63 b	43.6 b	29.6 b	5.6 b	0.21 a	30.1 c	6.4 b
CDC Golden	245 a	3.72 a	45.1 ab	29.0 c	8.6 a	0.20 ab	41.3 a	9.6 a
CDC Meadow	247 a	3.77 a	45.5 a	29.2 c	8.4 a	0.20 b	42.9 a	9.2 a
CDC Sage	220 b	3.61 b	43.3 b	29.9 a	5.8 b	0.19 c	35.3 b	7.0 b

Control: 24/18 °C day/night temperature and watered 100% ET; Heat: 35/18 °C day/night temperature and watered 100% ET, Drought: 35/18 °C day/night temperature and watered 40% ET; Combined: 35/18 °C day/night temperature and watered 100% ET. ET: evapotranspiration.

6.3.2 Evapotranspiration

Evapotranspiration (ET) was affected by treatment, cultivars and by the treatment x cultivar interaction (Table 6.1). No significant difference of ET was observed between the control and heat; or between drought and the combined stresses (Table 6.2). The cumulative ET under drought and combined stresses was lower than both control and heat treatments (Table 6.2). Peas under heat stress had a greater ET than the control during the first three days of the treatment duration; after this ET declined substantially (Figure 6.4A). Similarly, the combined stress had a greater ET than drought stress for the first three days, but smaller ET than drought from day four and afterwards. Cultivars varied in their response to the different stress treatments. Compared to the control, ET of Cooper and CDC Golden was not affected by the heat treatment. However, CDC Meadow and CDC Sage had a 5% increase and decrease in ET, respectively, in heat. Such an increase in ET for CDC Meadow suggested that enhanced ET could be one of its heat avoidance mechanisms. Evapotranspiration was negatively correlated with LT under control and heat stress conditions but not under drought and combined stress (Figure 6.5). CDC Meadow had the highest and lowest ET under heat and combined treatments respectively, suggesting this cultivar may have heat, but not drought, tolerance.

Table 6.3. Main effects of environment and cultivar on stem thickness, plant height, reproductive stem length, reproductive nodes and total nodes. Data are the mean of levels of environments and cultivars, and the means with a common letter within each column under each trait were not different at $P < 0.05$. N = 16 for stress regimes, and N = 16 for cultivars.

Effects	Flowering duration (days)	Stem thickness(mm)	Plant height (cm)	Reproductive stem length (cm)	Reproductive nodes	Total nodes
Environment						
Control	17.8 a	4.06 a	82 a	17.5 a	7.3 a	27.8 a
Heat	13.1 b	3.97 a	79 b	13.5 b	6.5 b	27.1 a
Drought	13.4 b	3.74 b	75 c	12.3 c	6.0 c	25.9 b
Combined	11.9 c	3.66 b	74 c	8.3 d	5.1 d	24.5 c
Cultivar						
Cooper	11.7 c	3.87 b	73 d	8.2 c	5.1 c	25.5 b
CDC Golden	15.8 a	3.66 c	81 a	15.5 a	6.9 a	26.5 ab
CDC Meadow	16.3 a	4.13 a	79 b	14.7 a	6.7 a	26.0 ab
CDC Sage	12.6 b	3.78 b	75 c	12.5 b	6.3 b	27.2 a

Control: 24/18 °C day/night temperature and watered 100% ET; Heat: 35/18 °C day/night temperature and watered 100% ET, Drought: 35/18 °C day/night temperature and watered 40% ET; Combined: 35/18 °C day/night temperature and watered 100% ET. ET: evapotranspiration.

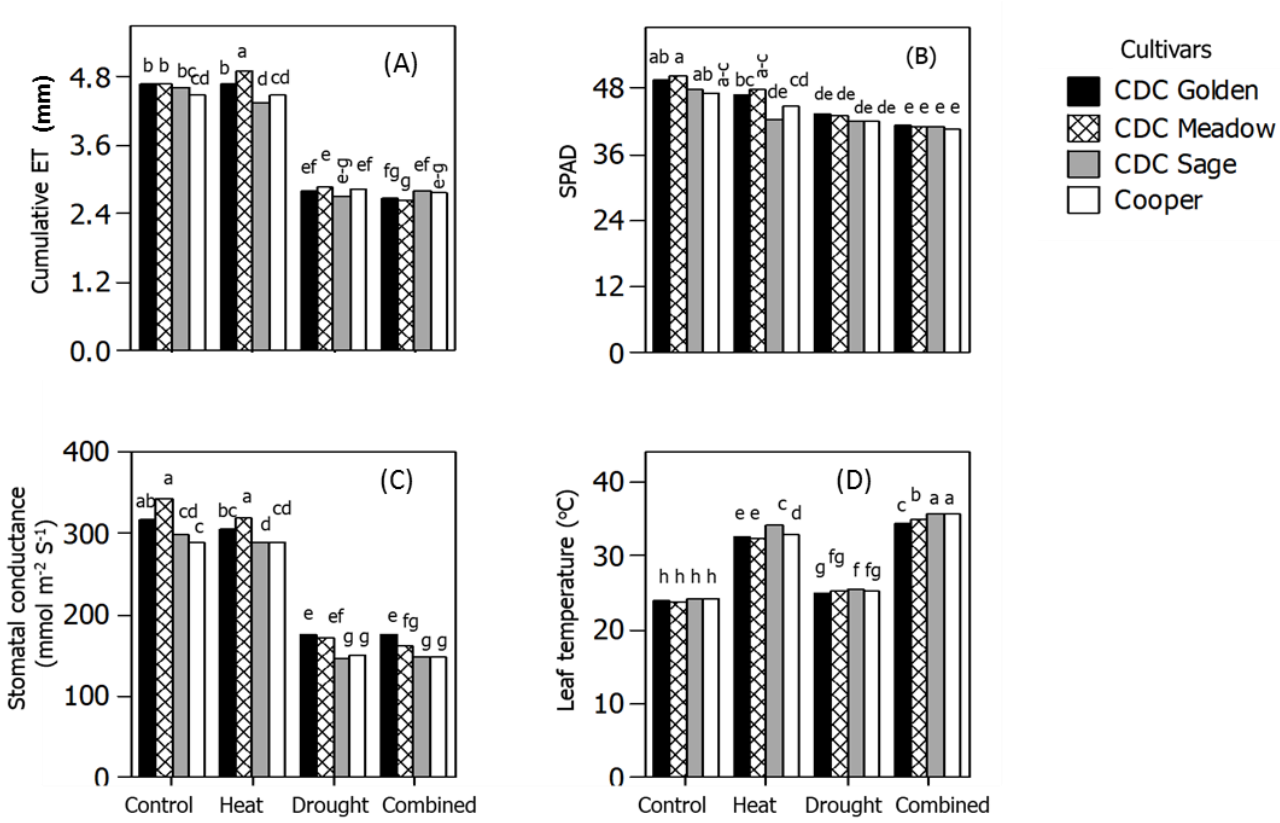


Figure 6.1. Environment x Cultivar interaction effects on physiological traits: cumulative ET (A) chlorophyll content estimation by SPAD meter (B), leaf stomatal conductance (C) and average leaf temperature (D) of four pea cultivars (CDC Golden, CDC Meadow, CDC Sage, and Cooper) grown under four stress conditions. Bars with similar letters within each panel are not significantly different at P <0.05. Each bar is the cultivar averaged over four replications and seven days (N = 28) per stress regime.

Note: Control: 24/18 °C day/night temperature and watered 100% ET; Heat: 35/18 °C day/night temperature and watered 100% ET, Drought: 35/18 °C day/night temperature and watered 40% ET; Combined: 35/18 °C day/night temperature and watered 100% ET. ET: evapotranspiration.

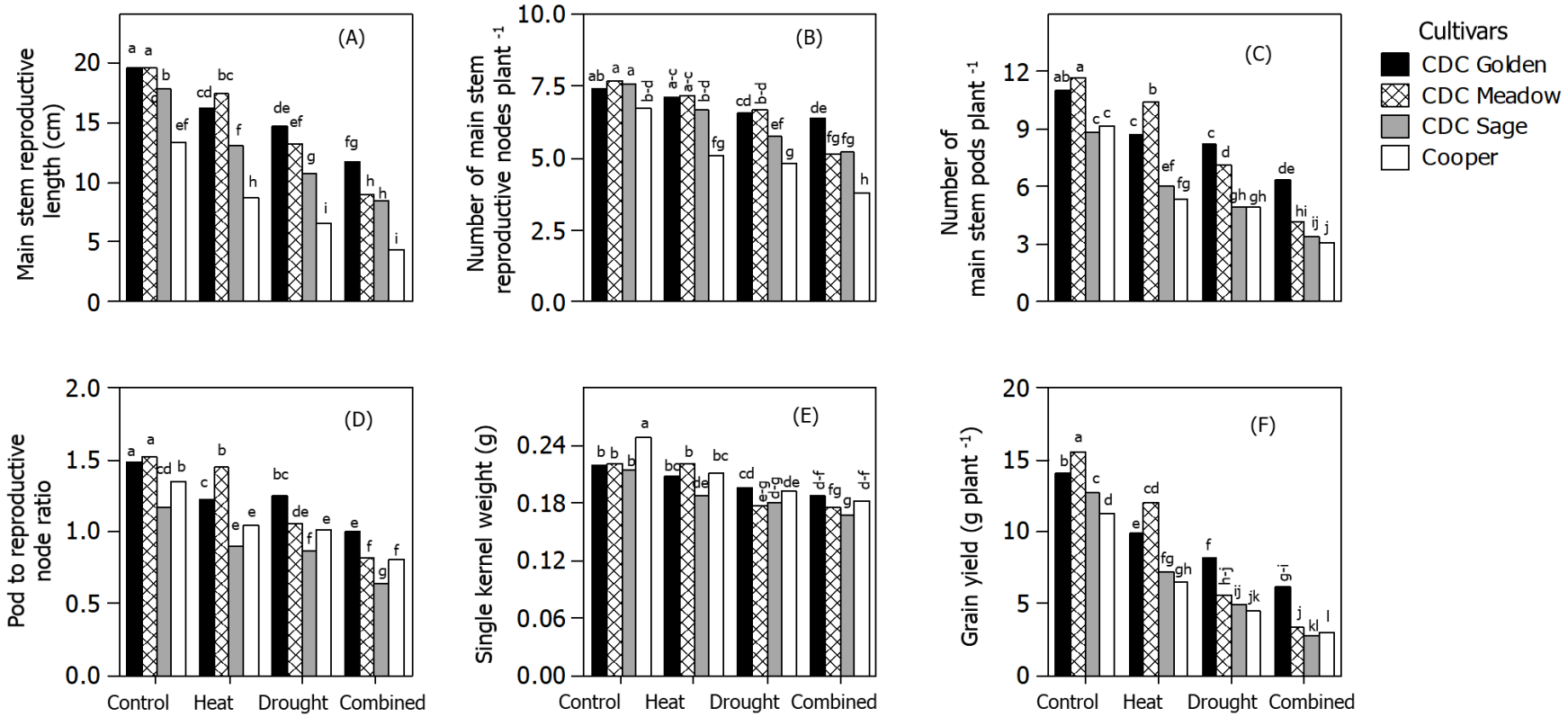


Figure 6.2. Environment x Cultivar interaction effects on growth and yield related traits, Reproductive stem length (A), Number of main stem reproductive nodes per plant (B), Number of main stem pods per plant (C), Pod set ratio (D), and Dry grain yield in gram per plant (E) of four pea cultivars (CDC Golden, CDC Meadow, CDC Sage, and Cooper) grown under control, heat, drought and combined stresses under controlled growth chambers. Bars with similar letters within each panel are not significantly different at P <0.05. Each bar is the cultivar averaged over eight replications per stress regime.

Note: Control: 24/18 °C day/night temperature and watered 100% ET; Heat: 35/18 °C day/night temperature and watered 100% ET, Drought: 35/18 °C day/night temperature and watered 40% ET; Combined: 35/18 °C day/night temperature and watered 100% ET. ET: evapotranspiration.

6.3.3 Leaf temperature

Compared to the control, the LT of peas under heat, drought and combined stresses was increased by 9, 1.1, and 11.2 ºC respectively (Figure 6.4C). First, this indicated that under high temperature stress, optimal water supply reduced LT by 2.2 °C. Second, decreased ET in drought and combined stresses caused a corresponding increase in LT (Figure 6.4A and B). In the control treatment, no significant difference was observed in LT of the four cultivars. Under heat stress, CDC Meadow and CDC Golden had lower LT than CDC Sage and Cooper, and under drought stress, CDC Golden had relatively cooler LT than all cultivars (Figure 6.1D). During the treatment duration, there was an overall increase of LT under all treatment conditions (Figure 6.4B) which might be partly associated with phenology and senescence of the plants.

6.3.4 Chlorophyll content estimation by SPAD meter

Chlorophyll content of pea was affected by cultivar and the stress treatments, but the stress regime by cultivar interaction was non-significant. The chlorophyll content was decreased by all stresses but the highest and least reduction was associated with the combined and drought stresses, respectively (Table 6.2). CDC Meadow and CDC Golden had greater chlorophyll content than Cooper and CDC Sage. The chlorophyll content of peas grown under control, heat and drought treatments increased during the first 10 days of the treatment duration, and then decreased at a greater rate than the control treatment. Chlorophyll content of peas grown under combined stress increased only during the first five days of the treatment duration then decreased afterward. Overall, chlorophyll content was degraded by all stress factors.

6.3.5 Plant growth and phenology

Stem diameter, plant height, and number of total nodes were significantly affected by cultivar and stress regime main effects, but not by the cultivar x heat regime interaction. Reproductive stem length and number of reproductive nodes were significantly influenced by cultivar and treatment main effect, and their interaction (Table 6.1). Heat and drought stresses, individually or in combination decreased plant height, reproductive stem length, total number of nodes, and reproductive nodes (Table 6.3). Reproductive stem length was decreased by 16, 32 and 52% due to heat, drought and combined stresses respectively (Table 6.3). Likewise, the number of reproductive nodes was decreased by 11.7, 20.8, and 33.8% due to heat, drought and combined

stresses, respectively (Table 6.3). Combined drought and heat had the greatest negative impact on growth and development of the plant reproductive parts followed by the drought. Heat stress did not affect stem diameter but drought and combined stresses reduced stem thickness.

The pea cultivars varied in many variables. CDC Meadow and CDC Golden had the widest (4.13 mm) and narrowest (3.66 mm) stem diameters respectively; and CDC Sage and Cooper had the largest (27.2) and smallest (25.5) number of total nodes, respectively (Table 6.3). Under all treatment conditions, Cooper had the shortest reproductive stem length and the smallest number of reproductive nodes (Figure 6.2A). CDC Golden and CDC Meadow had similar reproductive stem length and number of reproductive nodes under all treatment conditions except in the combined stress where CDC Golden had superior performance over CDC Meadow (Figure 6.2A and B). But, compared with the control, reproductive stem length of CDC Golden, CDC Meadow, CDC Sage, and Cooper were decreased by 18, 11, 27 and 35%, respectively, by the heat stress. The corresponding decrease due to drought stress was 25, 32 40, 51%; and the combined stress reduced reproductive stem length by 40, 54, 52, and 68%, respectively (Figure 6.2A). Similarly, the reproductive node number for CDC Golden, CDC Meadow, CDC Sage and Copper decreased by 4, 6, 12, and 25%, respectively, due to heat stress, and the corresponding decrease due to drought stress was 11, 13, 24, 29%, and the reproductive node number decrease in the combined stress was 14, 33, 31, and 44% respectively (Figure 6.2B). Node formation was less affected by the stress treatments, but internode length was shorter. CDC Meadow was less sensitive to heat stress, but its sensitivity was increased under drought and combined stresses. Compared to the control, both drought and heat had a 25% reduction in flowering duration with no significant difference between them, but their combined occurrence led to a 33% reduction in the flowering duration (Table 6.3).

6.3.6 Seed yield and components

Seed yield, pod number, pod set ratio, and seed size of the four pea cultivars under heat, drought and combined stress is shown in table 6.3. Seed yield, pod number per plant, and pod set ratio were significantly affected by cultivars, environment and cultivar x environment interaction (Table 6.1). Combined stress had the highest adverse effect on all yield related traits followed by drought stress; for example, pod number reduced by 23, 38, and 57% due to heat, drought and

combined stresses, respectively, and the corresponding decrease for seed yield was 33, 56, and 71% (Table 6.2).

Table 6.4. Percentage of pea cultivars pod number and seed yield loss under heat, drought and combined stresses relative the non-stressed control condition. Each data point is averaged over four replications.

Trait	Stress	Percentage decrease compared to the control			
		CDC Golden	CDC Meadow	CDC Sage	Copper
Number of pods	Heat	21	11	32	42
	drought	26	39	44	46
	Combined	42	44	61	66
Seed yield	Heat	30	23	43	42
	drought	42	64	61	60
	Combined	56	78	77	73

Control: 24/18 °C day/night temperature and watered 100% ET; Heat: 35/18 °C day/night temperature and watered 100% ET, Drought: 35/18 °C day/night temperature and watered 40% ET; Combined: 35/18 °C day/night temperature and watered 100% ET. ET: evapotranspiration.

The four pea cultivars response was different under each stress conditions. CDC Golden had greater yield performance than the other three cultivars under drought stress, and CDC Meadow had greater performance under heat stress than the other three cultivars (Table 6.4). Compared to pod number or seed yield, seed size was less affected, and the decrease in seed size was within the range 0-28% in which Cooper and CDC Meadow had the highest and zero reduction respectively (Figure 6.2E). Under control and heat stress, CDC Meadow had superior grain yield and yield components over other cultivars grown in similar conditions. Under drought and combined stresses CDC Golden had greater yield than other cultivars. CDC Sage and Cooper performed consistently lower under the stress treatments. Pod set ratio for all cultivars decreased due to heat, drought, and combined stresses. Combined stress had the greatest influence on pod set ratio, whereas heat stress had relatively less effect. Under heat stress, CDC Meadow had the greatest pod set ratio of all cultivars. CDC Sage consistently had the lowest pod set ratio under all treatments. Under drought stress CDC Golden had greater pod set ratio than the other three cultivars.

Correlation tests showed that seed yield variation was highly associated with pod number per plant (r = 0.92), seed number per plant (r = 0.97), and pod set ratio (r = 0.85). The correlation coefficient between seed yield and seed size was smaller (r = 0.65), and there was no significant correlation between seed number per pod and seed size (Figure 6.3). These results suggest while pod number per plant, pod set ratio, and seed number per plant could indicate seed yield, but seed size could not. Growth parameters associated with reproductive parts, such as main reproductive stem length and reproductive node number were positively associated with seed yield (Figure 6.2).

6.5 Discussion

Concurrent drought and heat stresses is a typical situation in semi-arid areas, and understanding the individual or interactive drought and heat effects, and plants' response pattern is essential to improve crop tolerance to multiple environmental stresses (Mittler 2006). My results indicated drought or heat had detrimental effects on most growth and seed yield traits, and the combined stresses exacerbated their impact. My results showed drought and heat had both unique and common effects (Prasad et al., 2011). While drought and combined stresses decreased stomatal conductance and evapotranspiration, heat stress enhanced the stomatal opening and led to increased stomatal conductance and evapotranspiration during the first three to four days (Figure 6.4A&D). Reduced stomatal conductance due to stomatal closure is an early plant response to drought stress as an avoidance mechanism by maintaining water in a plant (Hsiao 1973; Khan et al., 2010), but it is associated with reduced photosynthesis, growth and yield (Beebe et al., 2013). Stomatal closing under the combined and drought stresses was associated with an increased leaf temperature mainly due to the inability of plants to cool themselves through transpiration (Alexieva et al., 2001). In contrast, heat stress with sufficient water was associated with a cooler leaf temperature as evidenced by a 2.2 ºC reduction compared to the combined stress. Interestingly, pea treated under combined and drought stress showed similar responses (Grigorova et al., 2011; Awasthi et al., 2014) in gs and ET, although the combined stress led to a high leaf temperature. I also noted that pea subjected to drought stress had 1 ºC higher leaf temperature than the control, suggesting that drought could lead to heat stress. Such an increase of leaf temperature was due to stomatal closure and the inability of plants to cool themselves through transpiration cooling (Khan et al., 2010).

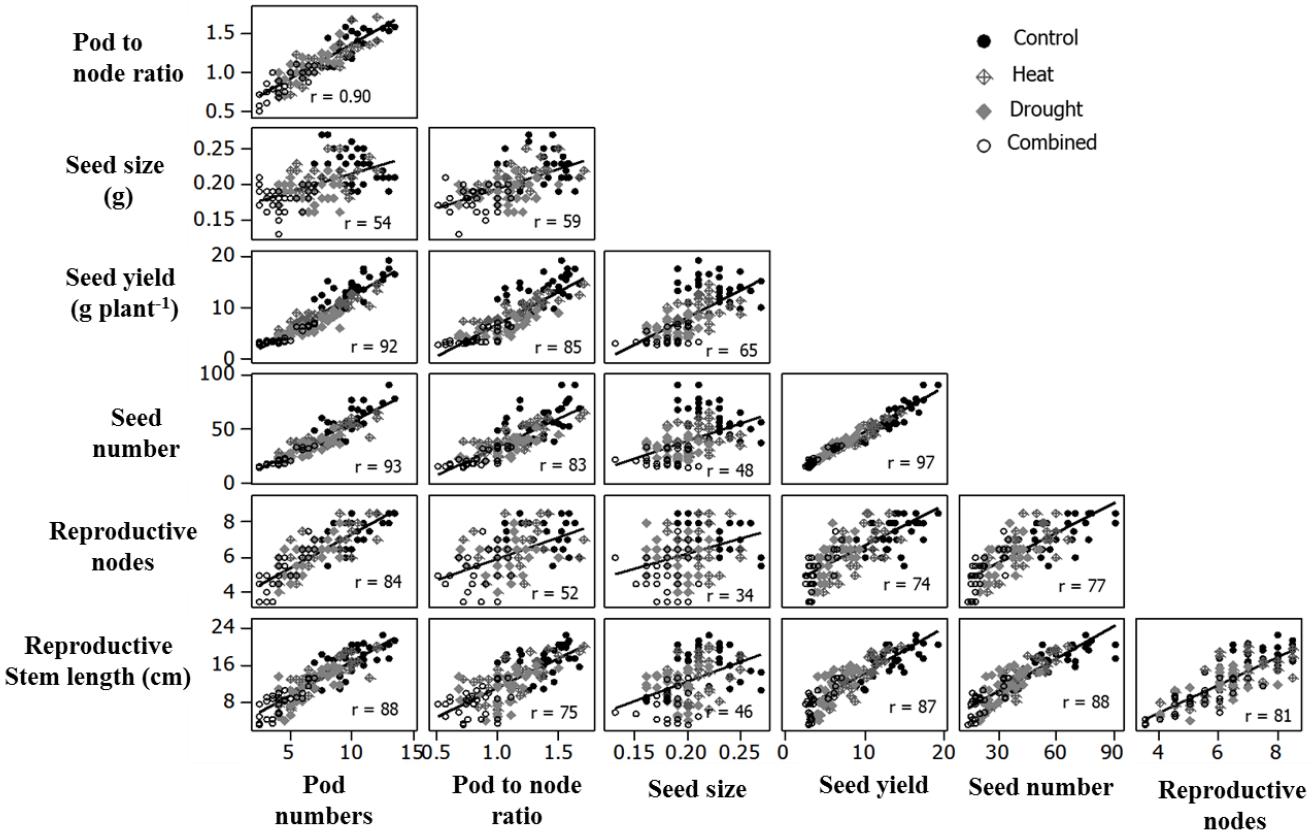


Figure 6.3. Matrix plots of the relationships among pod numbers, pod to node ratio, seed size, seed yield, seed number, reproductive nodes, and reproductive stem length of pea grown under various stress conditions in controlled growth chambers. The correlation coefficient (r) indicates the strength of the relationship. N=64.

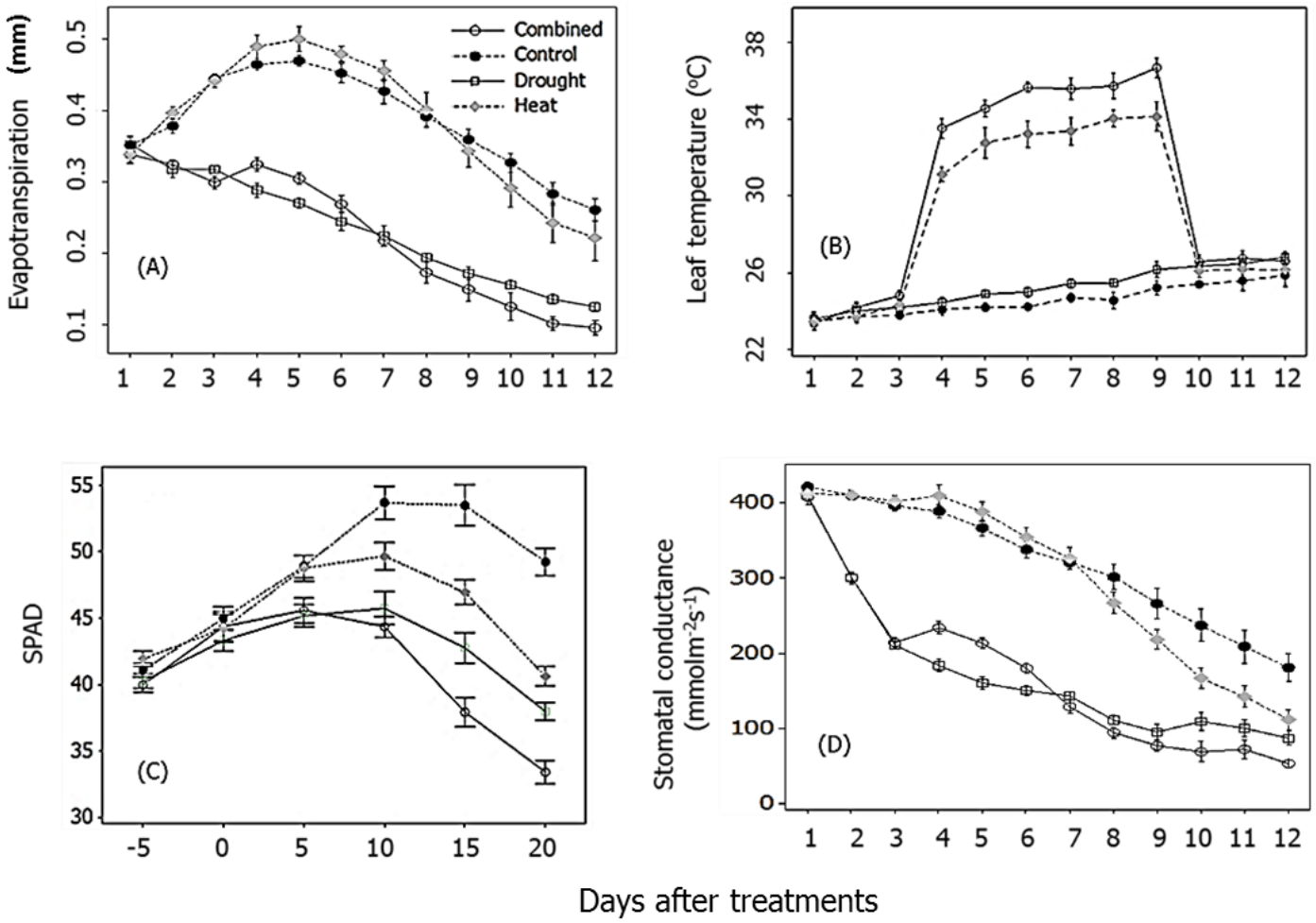


Figure 6.4. Evapotranspiration (a), leaf temperature (b), and chlorophyll concentration (c), and stomatal conductance response of pea grown under various stress conditions in controlled growth chambers. Each data point is averaged over 16 measurements (four cultivars and four replications). Error bars are standard errors of means (N = 16).

Heat stress almost always had a smaller effect on pea growth parameters than drought or combined stresses, indicating heat stress was moderated by moisture availability and transpiration cooling (Figure 6.5E). Heat stress did not reduce stem diameter, but drought alone and its co-occurrence with heat significantly reduced stem diameter. Reduction of stem diameter due to drought or combined stress was likely associated with a decrease in cell turgidity and stem shrinkage, in response to changes in stem water status (Klepper et al., 1971). Drought also alters stomatal movement and transpiration which directly governs plant growth, organ expansion, and biomass (Figure 6.4A and D). Alexieva et al (2001) demonstrated that reduction of leaf relative water content was one of the major causes for growth reduction under drought stress (Hsiao 1973). Prasad et al. (2008) indicated severe heat stress decreased stem length resulting in reduced growth and plant height. During treatment, the leaf temperature increase under the control and heat treatments was low compared to the corresponding increase under drought and combined stresses, suggesting the negative impacts of heat stress due to high air temperature can be mitigated by an optimal water supply.

The results from this study showed pod set ratio, seed size, and seed yield were affected by heat and drought, and the their combined occurrence had the greatest impact. Studies carried out on pea heat stress showed an overall reduction in yield and yield parameters due to increased air temperature beyond a certain threshold (Bueckert et al., 2015; Jiang et al., 2015). Under controlled environments, pea yield reduction was evident only after relatively high (> 31 ºC) temperature (Jeuffroy et al., 1990). Heat stress with sufficient water supply resulted in plants with greater plant height, pod numbers, and seed yield than the combined stress, suggesting the impact of heat stress in pea can be moderated by sufficient water supply.

Drought stress almost always had greater impact on pea growth and yield than heat stress. Although pea is relatively tolerant to moisture stress, studies conducted on drought stress alone revealed linear reduction in seed yield as the soil moisture depleted beyond a certain threshold (Martin and Jamieson 1996; Daryanto et al., 2015). Under all stress conditions, the most sensitive pea yield component was pod and seed numbers per plant, both having strong correlations with grain yield (Figure 6.3) suggesting drought, heat or their combination lead to ovule and pod abortions. Similar findings were seen from several studies conducted on pea under heat and drought stresses (Lambert and Linck 1958; Guilioni et al., 2003). Pod set relies on functioning male and female floral components, which are sensitive to heat stress and drought (Jiang et al.,

2015). In all yield parameters, the effect of heat stress alone was relatively milder compared to drought and combined stresses, inferring that the maximum threshold temperature affecting pea seed yield and its components can be increased by moisture availability.

Compared with the control, pod numbers were reduced by 25, 43, and 63% when cultivars were subjected to heat, drought and both stresses combined, respectively. Heat and the combined stress differed in effects, which suggests the greatest impact in the combined stress is from the drought component (Awasthi et al., 2014). My result suggested under high temperature, optimal water availability enhanced yield in a controlled environment. Similarly under field conditions, Bueckert et al (2015) indicated that precipitation increased pea yield in hot years. Such results strongly suggest that irrigation can mitigate heat stress.

Pea cultivars differed in sensitivity and response to each stress. Under heat stress, CDC Meadow had the highest gs, ET, lower leaf temperature coupled with greater pod number, pod set ratio and seed yield followed by CDC Golden. The greater stomatal conductance and evapotranspiration of CDC Meadow, coupled with the least leaf temperature, suggests the cultivar has an ability to avoid heat stress. Several studies showed low leaf and canopy temperature linked to high yield (Idso et al., 1982). Under drought and combined stresses CDC Golden had superior performance for yield traits. These results clearly suggest CDC Meadow is a heat tolerant cultivar and CDC Golden is a cultivar with intermediate tolerance to both drought and heat stresses. Cooper and CDC Sage performed consistently lower for most traits suggesting these cultivars are more sensitive to environmental stresses applied individually or in combination. CDC Meadow had a thick stem diameter which might hold water for longer time, CDC Golden had a thin stem and relatively small leaf size. An early study on cotton by (Klepper et al., 1971) indicated that stem diameter had a positive correlation with leaf water potential, and hence with lower leaf temperature. The leaf temperature difference demonstrated heat stress was mitigated by evaporative cooling.

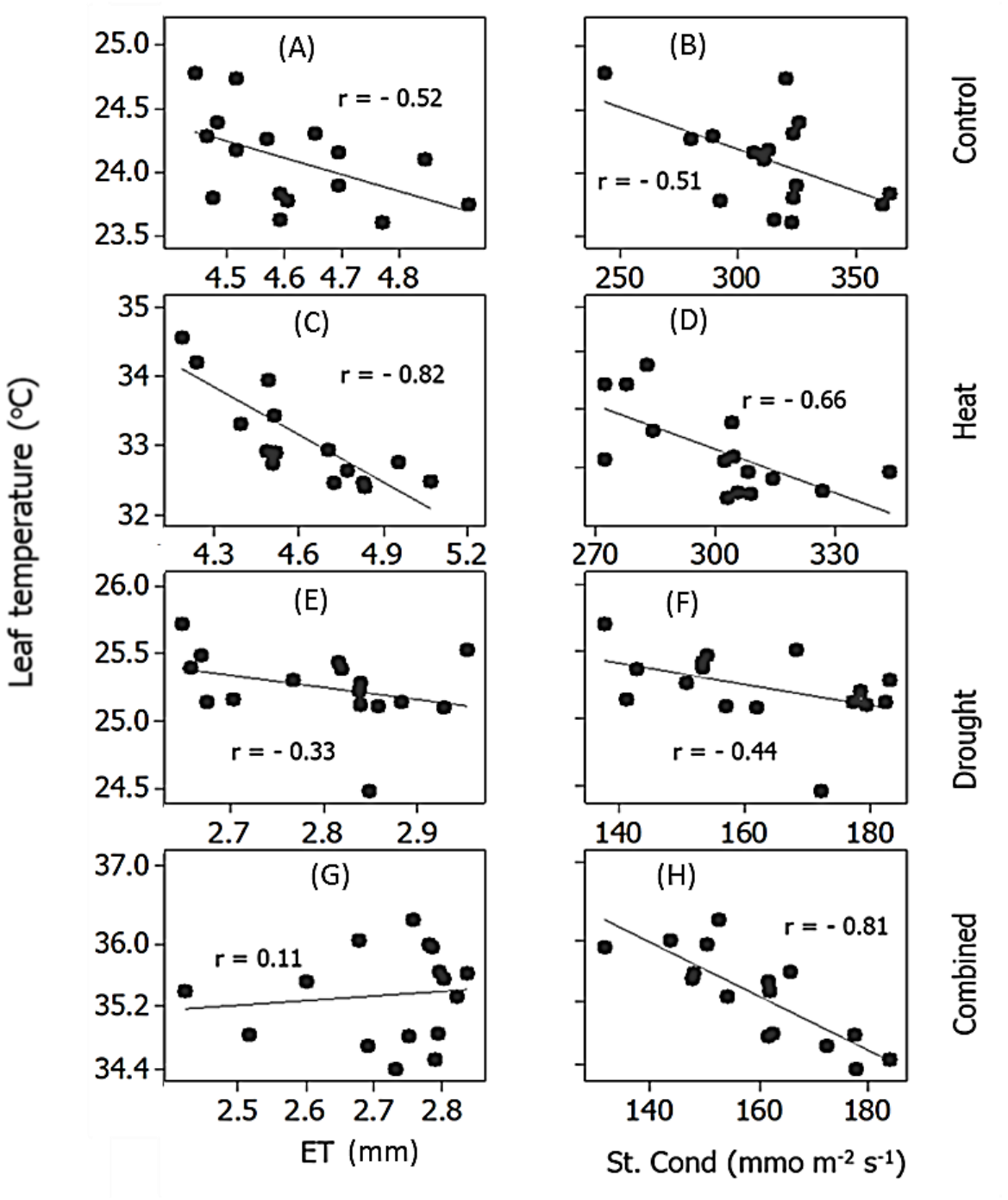


Figure 6.5. Scatter plot showing correlation between leaf temperature and various physiological measurements of pea grown under different stress conditions in controlled growth chambers. Leaf temperature negatively correlated with cumulative evapotranspiration and the strength of correlation varied by stress treatment type, and consistently strong correlation was seen under heat stress treatment. Each symbol is a cultivar averaged over four replications per each stress regime. ET= evapotranspiration, St.Cond = Stomatal conductance.

6.5 Conclusion

Drought or heat negatively affected pea physiological process, growth and yield; and their co-occurrence intensified the impacts and had additive effect for the growth and yield parameters. Each stress mostly had a unique effect on the physiological processes. Stomatal conductance and ET decreased due to drought and combined stresses, however these traits were either unaffected or slightly increased due to heat stress. Although all stresses increased the leaf temperature, abundant soil moisture enabled the heat treatment to decrease leaf temperature by 2.2 ºC compared with pea under the combined stress. Evapotranspiration and stomatal conductance were associated with lower leaf temperature under heat stress. Seed yield was strongly associated with pod total seed numbers but the association with size was low. Overall, concurrent drought and heat had the most detrimental impact on pea growth and yield followed by the drought stress. An optimal soil water supply can therefore moderate the impacts of heat stress. Pea cultivars have various degree of tolerance to the different stress regimes. Generally, CDC Meadow was heat tolerant, and moderately tolerant to drought, but sensitive to the combined stresses. CDC Golden was moderately tolerant to all stress conditions. CDC Sage and Cooper were sensitive to environmental stresses from the four cultivars used in this study.

Transition section between Chapter 6 and Chapter 7

Effects of individual and combined stresses of heat and drought on physiological growth and yield performance of four pea cultivars were investigated in Chapter 6. Stomatal conductance and cumulative evapotranspiration decreased due to drought and combined stresses, but not due to heat stress. Under the heat stress, optimal water supply reduced leaf temperature by 2.2^{o}C. The pea growth and seed yield traits decreased due to heat or drought, and their combined occurrence exacerbated the impact. Drought and combined stress effects had a similar pattern although the combined stress was most detrimental. CDC Meadow was tolerant to heat stress but not to the combined stress. CDC Golden was moderately tolerant to both heat and drought stresses. However, reports on pea threshold temperature for yield reduction vary widely and the objective of the experiment in Chapter 7 was to determine threshold air temperatures for heat stress failure for basic plant responses such as leaf stomatal conductance, pigment concentration, plant growth, and seed yield and to partition effects of leaf development stage from the heat stress responses.

CHAPTER 7. VEGETATIVE STAGE THRESHOLD TEMPERATURE, AND LEAF DEVELOPMENT STAGE ASSOCIATION WITH HEAT STRESS IN FIELD PEA

7.1 Introduction

Global ambient air temperature is increasing steadily at a rate of 0.3 °C per decade (Lobell and Gourdji 2012) which will severely impact yield of many crop species (Hatfield et al., 2011). Cool-season crops such as pea, lentil and chickpea are more sensitive to heat stress, and a daily maximum temperature 25-32 °C is considered as the upper limit for significant yield loss (Lambert and Linck 1958; Ibrahim, 2011; Kaushal et al., 2013; Bueckert et al., 2015). In pea, transient heat shock or a persistent increase in ambient air temperature leads to impaired photosynthesis, shortened flowering duration and accelerated senescence, flower and pod abortion, and consequently to a remarkable yield loss of up to 70% (Lambert and Linck 1958; Nonnecke et al., 1971; Pumphrey and Ramig 1990; Bueckert et al., 2015). Identifying air threshold temperatures would provide a starting point for assessing temperature related risks and immediate crop management options, particularly in a heat-sensitive crop like pea.

While pea's heat sensitivity has been extensively studied since 1950s (Lambert and Linck 1958; Karr et al., 1959), reports have been inconsistent in the threshold temperatures that result in yield reduction, warranting research and clarification before the crop can be improved. Lambert and Lick (1958) indicated 27-32 °C air temperature reduced yield, and they emphasized that longer exposure duration exacerbated yield loss. Nonnecke et al (1971) reported continued growth at 27/17 °C day/night temperature under a controlled environment resulted in 50-70% yield reduction. Stanfield et al (1966) reported pea yield reduction started when air temperature reached 16 °C and beyond, which is a relatively low threshold temperature. Ridge and Pye (1985) found that every 1 °C increase in air temperature during flowering resulted in a 0.6 tonnes hectare 1 yield reduction. Pumphrey and Ramig (1990) reported 25.6 °C as the maximum threshold temperature beyond which yield started to decline, and the decline became exponential beyond 27 °C. Recently, Sadras et al (2012) concluded mean air temperature for grain yield reduction during the reproductive phase was 25 °C. Bueckert et al (2015) reported 28 °C as a threshold temperature for yield reduction under short-season dryland conditions, which is a relatively higher threshold than earlier studies. In contrast, under controlled environments Jeuffroy et al (1990) found that pea yield

reduction was evident only after 31 °C, and Jiang et al (2015) found that 36 °C reduced pollen germination and pollen tube length.

The pea threshold temperatures from the above studies ranged from 16-28 °C under field conditions and 27-36 °C under controlled environments, clearly indicating that non-temperature factors contribute to the threshold temperature. Heat stress is often confounded with drought, and heat exposure may occur at different plant development stages, and not all growth stages have similar sensitivity to heat. For example, in addition to heat stress, canopy and leaf temperatures may change in response to drought and the plant development stage, expanding leaves usually have higher leaf temperature than fully expanded leaves (Cure et al., 1989; Marias et al., 2017). Together, identifying threshold temperatures for a range of physiological and growth processes, and finally yield reduction, can then be used to screen pea germplasm for more heat resistant material to breed for more climate robust crops. Precisely controlled environment helps to detect effect of the development stage from the effects of the ambient air temperature.

Most published heat threshold studies focused on reproductive stages with little information on vegetative threshold temperatures. Moreover, threshold temperature reports from field studies vary widely because field studies lack appropriate control over other contributing factors. My first objective was to determine threshold air temperatures for heat stress failure for basic plant responses such as leaf stomatal conductance, pigment concentration, plant growth, and seed yield. My second objective was to compare sensitivities during late vegetative growth prior to flowering for expanding, expanded and senescing leaves at the control temperature. My third objective was to look at the daily response of stomatal conductance and leaf temperature of upper expanded leaves to a 7-day cycle of heat stress.

I hypothesized that pea has different threshold temperatures for the various development stages and growth processes. Plants grown under controlled environments with sufficient water supply would be expected to have higher threshold temperatures than would be expected under field conditions. Finally, leaves at different development stages vary in their sensitivity and heat response as they differ in wax pigment and wax concentrations and degree of heat exposure.

7.2 Materials and Methods

7.2.1 Plant materials

In this study two experiments were conducted under similar management conditions, using two pea cultivars, CDC Meadow and CDC Sage, under controlled growth chambers. Both cultivars had semilealfess leaves, upright growth habits, and white flowers. CDC Meadow has some heat tolerance while CDC Sage is more stress sensitive based on heat tolerance traits including cool canopy, less flower and pod abortion, and seed yield. Days to flowering for CDC Sage and CDC Meadow were 42 and 36 days, respectively. To achieve similar flowering dates and phenological stages during treatment durations, CDC Sage was seeded one week earlier than CDC Meadow.

7.2.2 Experiment 1: Temperature regime

This experiment covers objective 1, to determine threshold air temperature for significant effect on stomatal conductance, growth, seed yield and related traits. The experiment had two treatment factors, cultivar and temperature regime, laid out as a split plot design, the cultivar as a main plot, and treatment regime as sub plot factors with four replications. The five temperature regimes were control of 24, 28, 31, 34 and 37 °C daytime temperatures. The experiment was repeated and for each run 40 pots were used (2 cultivars x 5 temperature regime x 4 replications).

7.2.3 Experiment 2: Leaf development stage

Development stage may confound the effects of heat stress, and Experiment 2 covers objective 2. This experiment also had two factors, the two cultivars and three leaf developmental stages (expanding, expanded, and senescing leaves) with four replications, and 24 pots in total (two cultivars x three leaf development stage x four replications). The experiment was designed as a split plot design, cultivar as main plot and development stage as sub plot factors. For leaf measurements, leaves (expanded, expanding, and senescing) were marked (tagged) and measurements were taken using these same three leaves for seven days.

7.2.4 Plant growth conditions

In both experiments, pot size was 3.8 L volume filled with Sunshine Gro® mix (Seba Beach, AB, Canada) and slow-release fertilizer (14-14-14, Type 100, Nutricote®, Brampton, ON, Canada). Each pot was seeded with three seeds, and resulting seedlings were thinned to two plants

one week after emergence. In addition to the slow release fertilizer, plants were supplied weekly with 500 mL per pot of half strength modified Hoagland's nutrient solution starting from three weeks after seeding until the end of the flowering duration. The growth medium moisture was monitored carefully by plants watering daily to 70% pot saturated soil media weight to avoid water deficit stress. Plants were grown at a control temperature of 24/18 °C day /night temperatures with 16/8 h photoperiod and irradiance of 450-500 μmol photons m^{-2} s^{-1} from cool fluorescent tubes in the growth chambers, and then transferred to each specific elevated temperature regime for seven days. After the 7-days heat treatments were done, pots were returned to the control regime until crop maturity.

7.2.5 Plant measurements

Measurements plant height, main stem reproductive length, number of reproductive and total nodes, and stem diameter; yield related traits including number of pods per plant main stem, number of seeds per pod, single seed size, seed yield, stomatal conductance, leaf temperature; and concentrations of chlorophyll, carotenoid, anthocyanin and wax. Description of how these measurements were taken is presented in 3.2.3, 4.2.2, 4.2.3, and 4.2.4 sections of this thesis. Stomatal conductance determined on fully expanded and mature leaf (second or third leaf down from the uppermost expanding leaf node) with a portable leaf porometer (Steady State Licor 1600, Li-Cor Inc, Lincoln, Nebraska, USA) on the abaxial (upper) leaf surface. Stomatal conductance measurements were taken every day around noon prior to pot watering. Leaf temperature was measured daily during the entire treatment duration using a digital infrared-red fever thermometer. Leaf temperature depression was calculated as the difference between the leaf and growth chamber temperatures. Plant height and node counts were measured daily during the treatment duration, and at physiological maturity. Pigment and wax concentrations were determined twice, just before the onset and after completion of the 7-day treatment according to the method described in section 4.2.2 - 4.2.4.

Number of reproductive nodes per plant was determined by counting the number of reproductive nodes on the main from the first flowering node to the last flowering node. Number of main stem pods per plant (pods) was determined by counting the total number of pods on main stem that contained at least one viable seed. Pod set ratio refers to the number of pods per reproductive node, and was calculated by dividing the total number of pods on the main stem by

the total number of main stem reproductive nodes. Stem diameter was measured using electronic digital calipers according to section 3.2.2. Reproductive stem length was determined as the length of main stem from the first reproductive node to the last reproductive node.

7.2.6 Data analysis

Statistical analysis was done on the variables plant development traits (plant height, main stem reproductive length, number of reproductive and total nodes, and stem diameter) yield related traits (number of pods per plant main stem, number of seeds per pod, single seed size, and seed yield) and physiological traits (stomatal conductance, leaf temperature, pigment and wax concentrations) using the Mixed procedure of SAS statistical software (version 9.4; SAS Institute, Inc., Cary, NC, USA). Analysis of variance (ANOVA) with the least significant difference (LSD) test ($P < 0.05$) was used. For leaf based measurements including stomatal conductance, leaf temperature, pigments and wax, the effects of the leaf development stage and temperature regimes were compared by combining them together. The effect of cultivar, heat regime and leaf development stage and their interaction were considered as fixed effects, and replication was considered as random effect.

7.3 Results

7.3.1 Effects of cultivar, temperature, and leaf development stages

At the end of the 7-day heat treatment, significant differences were observed between the cultivars, among the temperature regime main factors, and leaf development stages for stomatal conductance, leaf temperature, chlorophyll a, chlorophyll b, carotenoid, anthocyanin and wax, and at physiological maturity, for plant height, pod numbers and seed yield. The 7-d duration elevated temperature regimes did not significantly affect seed size at maturity. The cultivar x temperature regime and leaf development stage interaction was non-significant for all traits except for leaf temperature depression, anthocyanin concentration, and seed yield (Table 7.1 and 7.2).

Table 7.1. Means of leaf temperature, stomatal conductance, pigments, and wax of two pea cultivars grown under various heat regimes in controlled growth chambers; and probabilities from analysis of variance (ANOVA) showing effects of cultivars, leaf development stage and temperature regimes on physiological, pigment, wax, growth and yield parameters. Means with a common letter within each column under each trait were not different at $P < 0.05$. For each data point, N = 56 for cultivar, and N =16 for DSHR.

Effects	**Leaf temperature depression (°C)**		**Stomatal conductance (mmol $m^{-2}s^{-1}$)**		**Pigment and wax concentrations (µg cm^{-2})**							
	DAY 1	DAY 7	Day 1	DAY 7	Chlorophyll		Carotenoid		Anthocyanin		Wax	
					stipule	petiole	stipule	petiole	stipule	petiole	stipule	petiole
Cultivar												
CDC Meadow	-1.85 b	-0.79 b	295 a	289 a	32.0 a	29.3 a	34 a	26.1 a	0.76 a	0.65 a	23.2 b	27.4 a
CDC Sage	-1.25 a	0.07 a	289 a	261 b	29.0 b	25.1 b	31 a	22.2 b	0.65 b	0.60 a	27.1 a	28.2 a
Development stage and heat regimes												
Expanding	-0.63 a	-0.87 c	269 d	286 abc	32.7 ab	24.0 a	40 a	31 a	0.91 b	0.7 ab	23.6 bc	25.2 cd
Senescing	-0.75 c	-0.95 c	278 cd	262 cd	23.8 c	20.2 abc	27 cd	23.3 b	1.16 a	0.62 bc	28.7 a	21.5 d
Control (Mature)	-1.78 c	-1.13 c	275 d	296 a	37.0 a	23.6 a	36 ab	30.2 a	0.73 c	0.62 bc	21.7 c	26.2 bc
28 °C	-1.18 b	-0.12 b	290 cd	287 ab	36.6 a	21.0 ab	37 ab	24.3 b	0.41 e	0.67 ab	25.4 a-c	28.8 abc
31 °C	-0.94 ab	0.03 b	324 a	280 abc	31.3 b	19.5 bc	31 bc	22 bc	0.49 de	0.55 cd	24.7 a-c	32 a
34 °C	-1.92 c	0.21 a	309 ab	270 bc	27.9 bc	18.0 bc	32 bc	20 bc	0.64 c	0.48 d	25 a-c	31.5 a
37 °C	-2.7 d	0.33 a	300 bc	242 d	24.3 c	16.5 c	22 d	18.1 c	0.62 cd	0.73 a	27.2 ab	29.7 ab
Significance												
Cultivar (C)	**	***	ns	*	*	**	ns	***	**	.	**	ns
DSHR	***	***	***	***	***	**	***	***	***	***	*	***
C x DSHR	*	*	ns	ns	ns	ns	ns	ns	*	**	ns	ns

***, **, *Significant at $P \leq 0.001$, 0.01 and 0.05, respectively. ns = non-significant at $P \leq 0.0$. DSHR = development stage and heat regime.

Table 7.2. Means of various growth and yield traits of two pea cultivars grown under various heat regimes under controlled growth chamber; and probabilities from analysis of variance (ANOVA) showing effects of cultivars, temperature regimes and their interaction on growth and yield parameters. Means with a common letter within each column under each trait were not different at $P < 0.05$. For each data point, N = 56 for cultivar, and N = 16 heat regimes.

Effects	**Plant height (cm)**	**Number of nodes**	**Number of pods**	**Seed size (g)**	**Seed yield (g plant^{-1})**
Cultivar					
CDC Meadow	80.6 a	26.7 a	10.8 a	0.22 a	11.3 a
CDC Sage	70.0 b	25.8 a	6.7 b	0.19 b	7.6 b
Development stage and heat regimes					
Expanding					
Senescing					
Control (Mature)	83.9 a	27.0 a	9.8 a	0.21 a	11.2 a
28 °C	81.6 a	26.5 a	9.3 ab	0.21 a	10.5 ab
31 °C	77.6ab	26.4 a	8.8 b	0.21 a	9.7 b
34 °C	74.3b	25.9 a	8.2 c	0.20 a	8.8 c
37 °C	70.0 b	25.2 a	7.1 d	0.20 a	7.5 d
Significance					
Cultivar (C)	***	ns	***	***	***
DSHR	**	**	***	ns	***
C x DSHR	ns	ns	ns	ns	*

7.3.2 Leaf temperature and stomatal conductance

During the 7-day heat treatment leaf temperature depression was gradually increased for all temperature regimes and leaf development stage treatments except for expanding leaves. However, the leaf temperature depression exceeded 0 only for 34 °C and 37 °C on the seventh date. The leaf temperature increase in 28 and 31 °C treated plants and senescing leaves was not significantly different from the control. The 34 °C and 37 °C-treated plants had a 2.3 and 3.1 °C leaf temperature increase, respectively which were significantly greater than the control. In contrast, the leaf temperature of expanding leaves decreased by 38% (Table 7.1). Results implied that pea was able to maintain cooler leaf temperatures than the ambient air temperature until air reached 34 °C. The two pea cultivars responded differently, and during the treatment duration the average leaf temperature of CDC Meadow and CDC Sage increased by 1.0 and 1.3 °C, respectively (Table 7.1). Leaf temperature depression was negatively correlated with the growth chamber air temperature for CDC Meadow but the correlation was positive for CDC Sage (Figure 7.5b).

Temperature regime treatments and leaf development stages also influenced stomatal conductance. The control and expanding leaves had a 4 and 10% increase, respectively, in stomatal conductance, while stomatal conductance decreased in the remaining treatments decreased during the 7-day treatment duration. The stomatal conductance decrease ranged from 6 to 16% for senescing leaves, and for 28 °C and 31 °C regimes. The stomatal conductance decrease was 20 and 26% for 34 °C and 37 °C regimes, respectively (Figure 7.1b). By the last day of the treatment, differences were observed in stomatal conductance between the control and the first two temperature regimes (28 °C and 31 °C), meaning that the threshold for stomatal function was influenced by exposure to 34 °C and higher. At the end of the treatment duration, CDC Meadow and CDC Sage decreased stomatal conductance by 10 and 15%, respectively. As air temperature increased, CDC Meadow cooled itself more quickly than CDC Sage, likely due to enhanced transpiration.

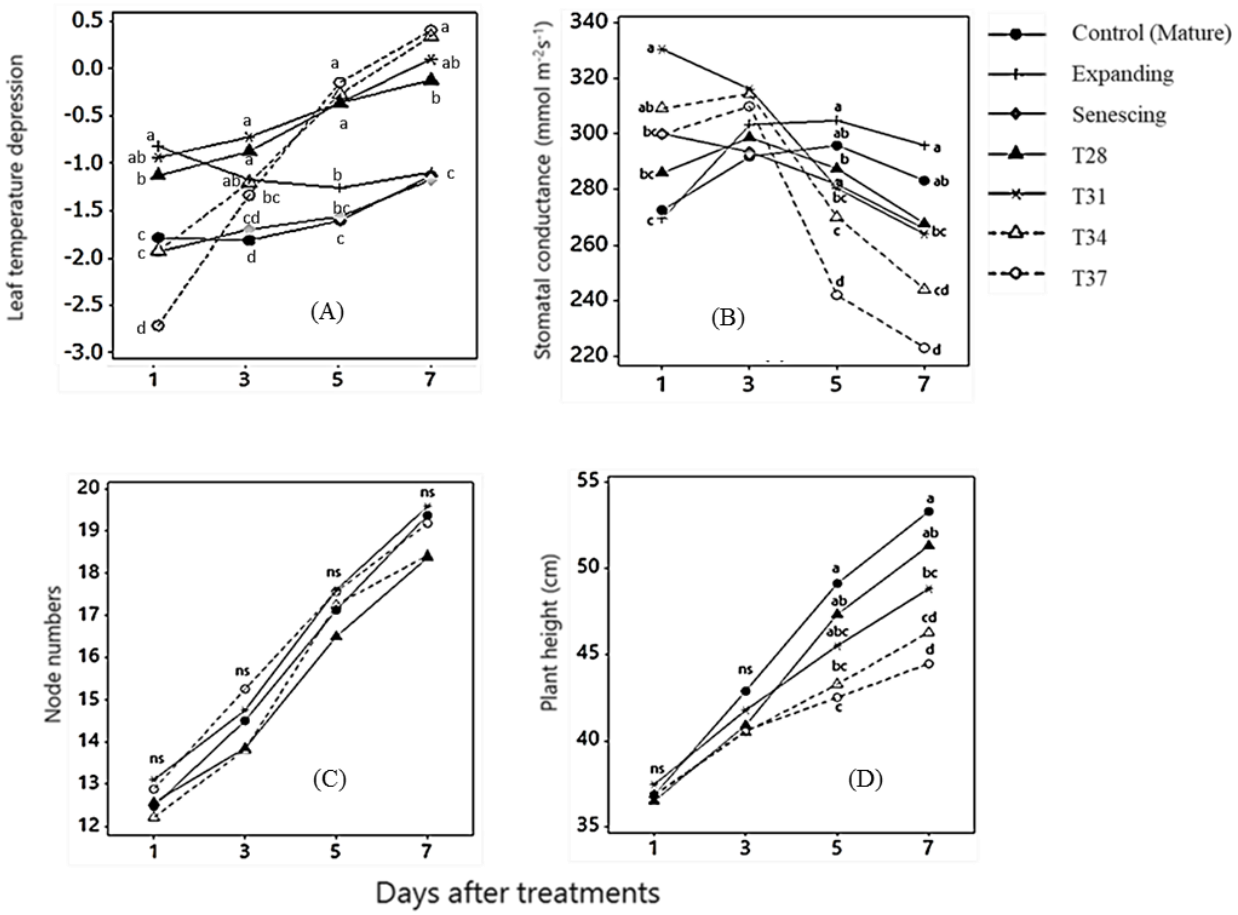


Figure 7.1. Effect of leaf development stage and temperature treatments on pea leaf temperature depression relative to the growth chamber temperature, stomatal conductance, node formation, and plant height during 7-days heat treatment in growth chamber. Leaf temperature depression was determined as the difference of the leaf temperature and the growth chamber temperature. Treatments followed by similar letters for each variable did not differ significantly ($P < 0.05$) for each treatment day. Each data point is averaged over eight replications and 2 cultivars ($N = 16$).

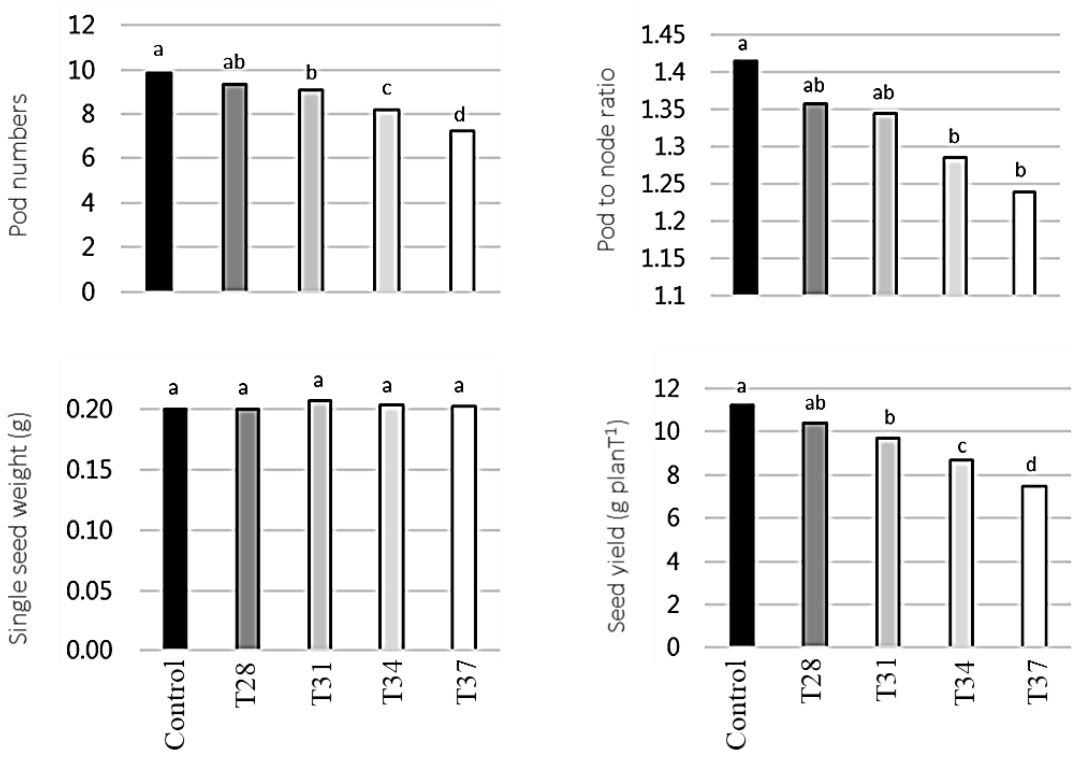


Figure 7.2. Effect of temperature regimes on final pod numbers, pod to node ratio, single seed weight and seed yield of pea grown under different temperature regimes in controlled plant growth chamber. Bars for each variable with similar letters do not differ significantly at $P < 0.05$. Each bar is averaged over eight replications and two cultivars ($N = 16$).

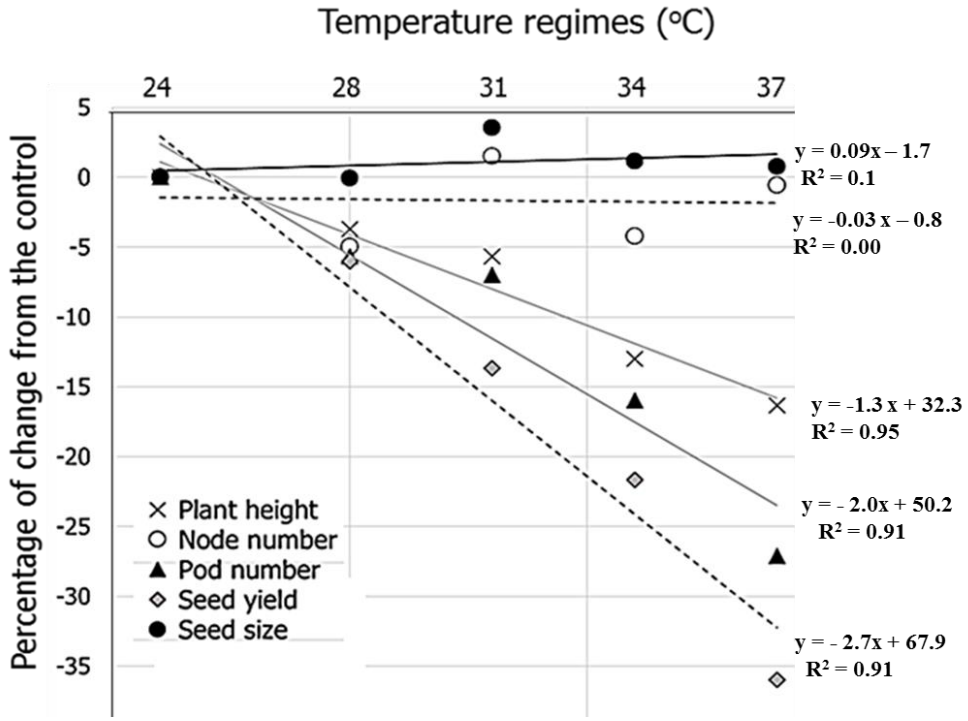


Figure 7.3. Percentage change in plant height, node number, pod number, seed size and seed yield relative to the control heat regimes for 7-day heat exposed pea plants. Plant height, pod number and seed yield were linearly reduced as daytime air temperature increased, but node number and seed size were unchanged. Each data point is averaged over eight replications and two cultivars (N = 16).

7.3.3 Pigment and wax concentrations

Stipule and petiole pigment and wax concentration varied between the two cultivars, among the five temperature regimes, and between the three leaf development stages (Table 7.1). Generally, the stipule and petiole chlorophyll, carotenoid and wax concentrations increased (30-60%) during the 7-day heat regime and leaf development stage treatments. However, the corresponding anthocyanin concentration was decreased (7-14%) except for the senescing leaves (Figure 7.4). After the 7-day heat treatment duration, while chlorophyll and carotenoid concentrations showed a decreasing trend with the increasing air temperature regime, the wax and anthocyanin concentrations had an increasing trend (Table 7.1 and Figure 7.4). Compared with the control, the chlorophyll concentration was decreased by 15.4% under 31 °C, which was identified

as a threshold temperature for significant chlorophyll loss, and the reduction was increased to 34.3% under the highest (37 °C) temperature regime. Similarly, senescing leaves had 35.7% less stipule chlorophyll concentration than the control. Carotenoid concentration mostly behaved like the chlorophyll concentration both for the stipule and petiole (Figure 7.4b and 7.4f). These results demonstrated that high temperature degraded green and photosynthetic pigments, and also as leaves became older, chlorophyll further degraded. In contrast, compared to the mature leaves (control), the expanding and senescing leaves respectively had 24.7 and 58.9% greater anthocyanin concentration (Table 7.1). CDC Meadow had greater stipule anthocyanin concentration than CDC Sage.

Compared with the control, 28 °C lead to a significant increase in stipule wax concentration. Also, the petiole wax concentration had an increasing trend for 24, 28, and 31 °C temperature regimes and then started to decline under 34 °C and 37 °C (Figure 7.4h). High temperature led to petiole wax degradation likely due to a melting effect of heat or interference with wax platelets. Interestingly, petioles had warmer temperatures than stipules by at least by 1 to 2 °C (data not shown). CDC Sage had significantly greater stipule wax concentration than CDC Meadow but both cultivars had similar petiole wax concentration.

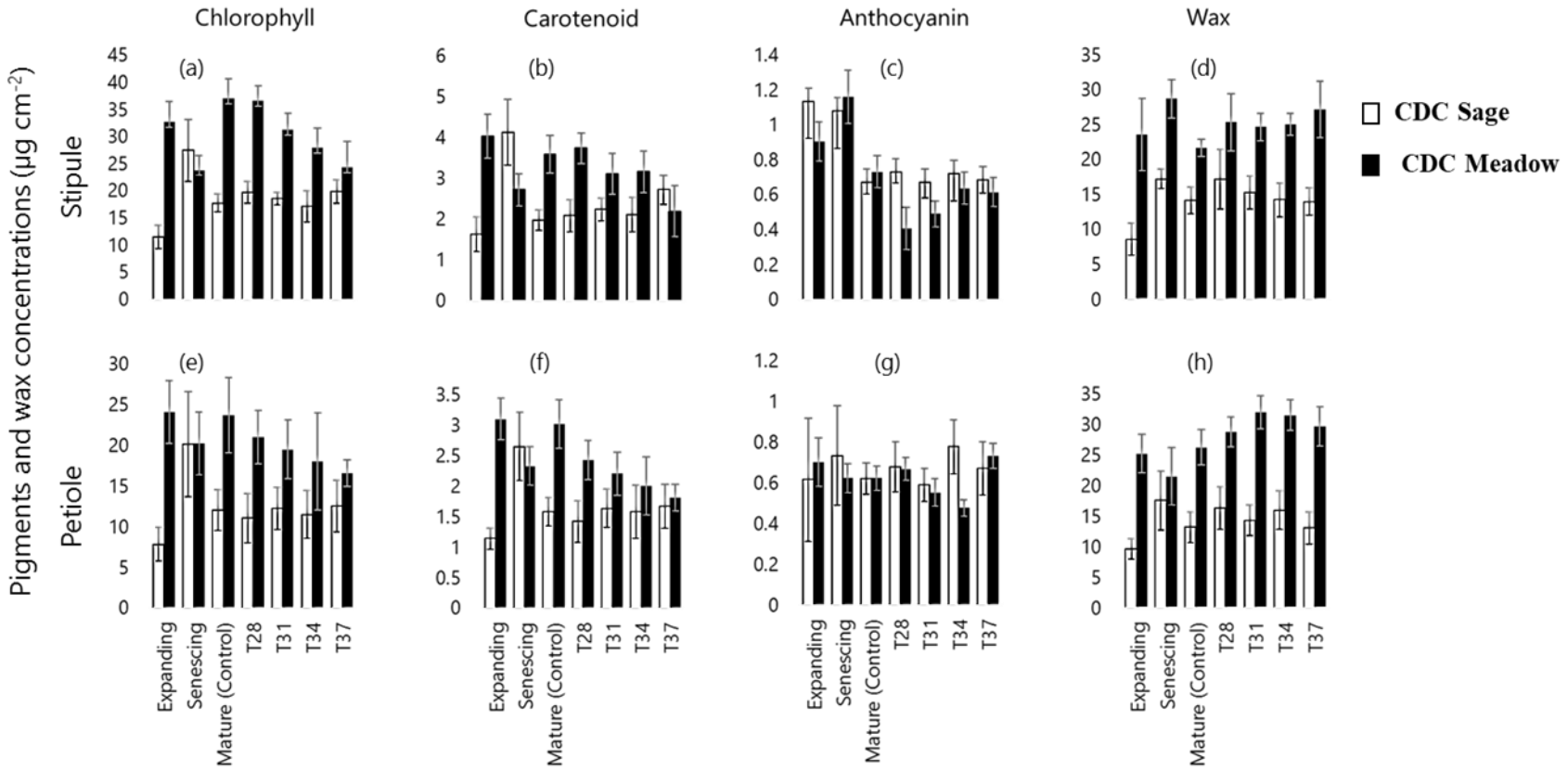


Figure 7.4. Effect of temperature regime and leaf development stage on stipule and petiole chlorophyll, carotenoid, anthocynanin and wax concentrations of pea grown under different temperature regimes in growth chamber. Each bar is a cultivar averaged over eight replications, N = 8, and error bars are standard errors of means.

7.3.4 Plant height and node count

Compared to the control treatment, cultivar height was lower after five days of heat exposure at 34 °C, or after 7-day of exposure to 31 °C (Figure 7.1D) compared to the control. At day 5 of the heat treatment, temperature regimes of 34 and 37 °C led to a 12 and 14% reduction in plant height, respectively, and at the end of the treatment duration the corresponding decrease in plant height was 13 and 17%, respectively (Figure 7.1). At physiological maturity, the plant height reduction from the control for 7-days heat treatment at 34 and 37 ºC was 13 and 17%, respectively (Figure 7.3). Therefore, threshold temperatures for heat stress were determined by both the air temperature regime and duration of exposure. A short duration of exposure to heat up to 31 °C did not affect vegetative growth, so 34 °C was taken as the threshold for significant decrease in plant height. Node numbers were not affected by the heat stress during the treatment duration, so a 7-day heat stress may not limit node formation, although there was a significant reduction in extension growth (plant height and internode length, Figure 7.3).

7.3.5 Yield and components

Yield and yield components (pod number and seed number) significantly decreased at 31 °C and beyond (Figure 7.2). Compared to the non-stressed control, pod number under treatments 31 °C, 34 °C, and 37 °C respectively decreased by 7.6, 16.5 and 26.3%, respectively, and the corresponding decrease for seed yield was 13.5, 22.5 and 33.4%, respectively. No significant decrease was observed for single seed weight (Figure 7.3).

The two cultivars generally had a contrasting nature for most traits described in this study. In most measurements CDC Meadow had superior performance over CDC Sage (Figure 7.4 and 7.5). Specifically, CDC Meadow had greater plant height, pod numbers, single seed weight, and pod to node ratio, seed yield and stomatal conductance than CDC Sage (Figure 7.5). Leaf temperature depression of CDC Meadow decreased with increasing air temperature, whereas the corresponding leaf temperature depression was not significantly influenced by temperature regimes in CDC Sage (Figure 7.5b). In contrast, the stomatal conductance of CDC Sage decreased with increasing air temperature but there was a slight increase in CDC Meadow (Figure 7.5c). A plausible explanation is that as the air temperature increases, CDC Meadow cools itself more by enhanced transpiration more than CDC Sage.

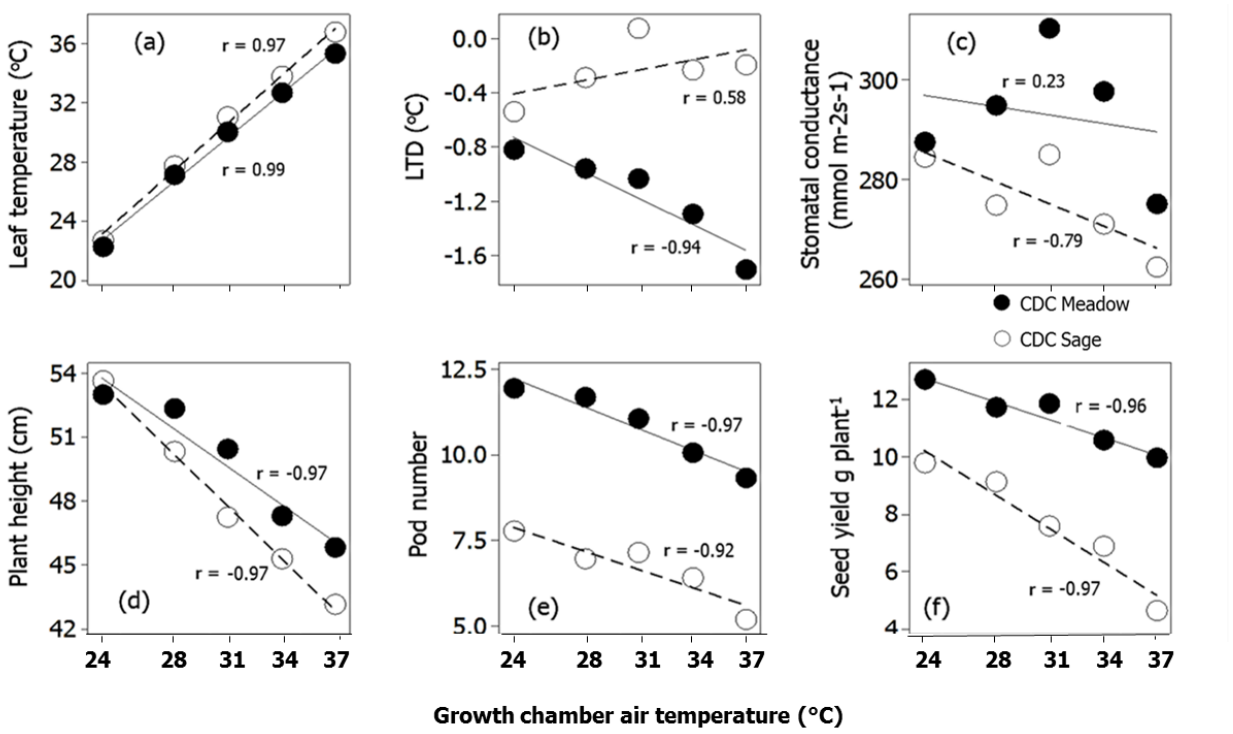


Figure 7.5. Response of two pea cultivars, CDC Meadow (closed circles) and CDC Sage (open circles) exposed to different temperature regimes (24-37 °C) in controlled plant growth chambers for seven days during late vegetative growth. Presented values are means (eight replicates) of plant parameters at each temperature regime. The relationship between the growth chamber air temperature and leaf temperature, leaf temperature depression, stomatal conductance, plant height, pod number per plant, and seed yield is presented in the individual panels (a–f).

7.4 Discussion

7.4.1 Controlled environment has a greater threshold

Heat stress has always been a major limitation in pea production (Pumphrey and Ramig 1990; Guilioni et al., 2003; Sadras et al., 2013; Bueckert et al., 2015). In this study, I exposed two pea cultivars to different daily maximum temperature regimes (24-37 °C) for seven days, with 18 °C constant night temperature. Also, impacts of leaf development stage were analyzed by employing leaf-based measurements on expanding (first node leaves), expanded (second or third node leaves), and senescing (seventh node leaves). My data showed 7-day exposure to 31 °C, or five days exposure to 34 °C, resulted in significant reductions of stomatal conductance, leaf temperature depression and plant height. Such reductions are mostly attributed to heat induced

inhibition of metabolic processes that involve enzymes and hormones, and limited gas exchange including stomatal conductance and photosynthetic rate (Rollins et al., 2013; Sehgal et al., 2017).

Our results demonstrated that a longer duration of exposure at relatively low temperature regime equally limited plant growth as a shorter duration exposure to high temperature (Wahid et al., 2007; Prasad et al., 2017). Under field conditions, 28 °C was the maximum threshold temperature reported for a significant pea yield reduction (Bueckert et al., 2015). However, I noticed that under a controlled environment, 28 °C led to only a 4% yield reduction compared with the non-heat stressed control, which was statistically non-significant. Instead, my results from the controlled environment had threshold temperatures above 31 °C for a significant negative influence on most measurements including pod numbers and seed yield, in agreement with two other controlled environment studies (Jeuffroy and Sebillotte 1997; Jiang et al., 2015)

Therefore, other factors such as soil moisture, solar radiation quality and receipt and evaporative demand likely contributed to the lower threshold temperatures reported under the field conditions (Jones and Rotenberg 2011; Bueckert et al., 2015). The huge range (15-28 °C) in threshold temperature reported over several decades from field studies likely arises from these non-temperature factors but contributed to plant abiotic stress. However, I noted that the stress response of different plant functions and processes varied. Node formation was little affected by heat stress even at the highest air temperature regime, which agrees with soybean results where heat stress during vegetative growth marginally affected node formation (Allen et al., 2018). In contrast, plant height and internode length were significantly affected from 31 to 34 °C (Table 7.1). My results also demonstrated that seed size was less affected by high temperature stress (Figure 7.2). Poggio et al (2005) reported that pea seed size was mostly unaffected by heat stress except for seed from the apical nodes. The heat treatments could have caused flower bud or seed abortion, and remaining seed filled to its genetically inherited seed size. In contrast, seed number, seed yield and pod number were significantly affected even at a relatively lower temperatures regime which could be attributed to flower, pod and ovule abortions (Guilioni et al., 1997; Poggio et al., 2005; Sadras et al., 2013; Jiang et al., 2015) when heat stress occurs in reproductive growth, specifically during early- to mid-flowering.

7.4.2 Vegetative stage heat stress leads to seed yield loss

Most pea heat stress studies mainly focused on the heat stress during the reproductive stage and information on vegetative stage heat stress threshold seldom exists. My results revealed heat stress during late vegetative stage significantly influenced pea growth and yield depending on air temperature regime and duration of exposure. As heat stress was prolonged, the difference among the temperature regimes became remarkably evident (Prasad et al, 2017). Though the plants were under the heat stress treatment during the late vegetation stage, in addition to the vegetative growth, yield and yield components were significantly reduced by the heat stress. In pea seed yield was positively correlated with plant height and internode length (Espósito et al., 2009). Vegetative-phase heat stress causes many physiological and metabolic alteration such as hormone imbalance and enzyme malfunctioning which consequently contributes to retarded growth and yield loss (Maestri et al., 2002; Barnabás et al., 2008; Reichler et al., 2009). The late vegetative stage prior to flowering appears to be most heat sensitive.

Plant pigment types and abundance are related to crop productivity. For example, leaf chlorophyll concentration is associated not only with photosynthetic activity, but also the progression of senescence due to stress, leaf aging, the plant lifecycle, and yield potential (Sehgal et al., 2017). Heat stress exposure above 31 °C for seven days during the vegetative phase led to reduced chlorophyll concentrations which directly reduced photosynthetic processes and retarded growth (Karim et al., 1999; Dutta et al., 2009).

7.4.3 Various plant processes have different threshold temperatures

Our data showed as the air temperature increased, there was an immediate response in stomatal conductance and leaf temperature depression. For the first few days conductance increased, and was associated with low leaf temperature depression, and the leaf was cooler than the growth chamber air temperature most likely due to transpirational cooling (Kashiwagi et al., 2008). Significant differences in leaf temperature depression and stomatal conductance were observed among the heat regimes from the first day of the heat treatment; here greater stomatal conductance and less leaf temperature depression were associated with the high temperature regimes. These results implied under sufficient moisture availability when pot watering is carefully controlled, stomatal cooling can serve avoid plant heat stress (Khan et al., 2007; Awasthi et al., 2014; Sehgal et al., 2017), at least at the beginning of a heat stress cycle. However, the longer the

stress persisted for over 5 days, plants gradually became stressed and leaf temperature depression rose rapidly while stomatal conductance was reduced (Figure 7.1B). The initial reduced leaf temperature depression was associated with a gradual leaf temperature increase. The later heat stress-induced escalation in leaf temperature was recently reported in lentil (Sita et al., 2017).

Compared to the control, significant chlorophyll and carotenoid concentrations reduction was observed after 7-day heat exposure at 31 °C and beyond whereas the corresponding wax and anthocyanin concentrations was significantly increased at 28 °C and beyond (Figure 7.4). Stipule and petiole had different threshold for the pigment and wax alterations. The chlorophyll loss was caused by decreased biosynthesis, heat induced pigment degradation, or both (Karim et al., 1999). Plant height and node numbers were not affected until the temperature reached 34 °C in controlled environment conditions. Pod numbers and seed had lower threshold temperatures.

7.4.4 Duration of exposure and leaf development stage influences threshold temperature

In this study I investigated the duration of exposure versus air temperature regime on two pea cultivars. Greater duration of heat exposure led to more damage and the difference between the control and the heat regimes increased with the number of days under the heat treatments. Marias et al (2017) showed longer heat exposure led to greater physiological damage and slower recovery. Prasad et al., (2017) indicated long exposures reduced harvest index of several field crops. At a constant 31 °C regime, heat stress was detected on day 7, but at 34 °C stress was detected earlier on day 5. My results revealed heat stress during late vegetative stage significantly influenced pea growth and yield depending on air temperature regime and duration of exposure. As heat was prolonged, the difference among temperature regimes became more remarkable which is in agreement with reports on wheat and legume crops (Prasad et al., 2017).

7.4.5 Heat sensitivity depends on leaf development stage

Leaf temperature was a primary indicator of stress in expanding leaves, but stomatal conductance response was smaller. Expanding leaves had less chlorophyll and cuticle wax but high anthocyanin concentration. Pigments and wax are involved in heat dissipation (chapter 4 and 5). Expanding leaves are also more sensitive to stress than expanded mature leaves and senescing leaves (Hsiao, 1973; Marias et al., 2015). My findings agrees with a report on coffee seedlings where expanding leaves were reported to be more sensitive to heat stress than fully developed

leaves, with decreased photosynthesis and less recovery from heat (Marias et al., 2017). Sensitivity of the expanding leaves could also be associated with the lesser wax concentration. Cuticle wax concentration of leaves is inversely related to leaf temperature, so leaves with more wax can dissipate more heat and lower leaf temperature (Sánchez et al., 2001; Jones and Rotenberg 2011).

7.5 Conclusions

The controlled environment had a greater threshold temperature than the field condition for similar growth and yield damage. I identified threshold temperatures that limit key physiological, growth and yield parameters of pea for the controlled environment. For plant height, node numbers, chlorophyll and carotenoid had high thresholds (≥ 34 °C) whereas yield related traits such as pod number and seed yield had relatively low (≤ 31 °C) thresholds. While node formation and seed size were unaffected by heat treatment by the end of the study, heat stress exposure during the late vegetative stage just prior to flowering reduced plant growth and seed yield.

Expanding leaves were more sensitive to heat stress and had a lower threshold temperature than mature and senescing leaves. Expanding and senescing leaves had greater anthocyanin concentration than matured leaves. Finally, duration of heat exposure was equally as important as the magnitude of the air temperature regime. Traits with the lowest (≤ 31 °C) thresholds should be targeted first in breeding programs to improve pea adaptation to warmer climates, specifically yield related traits including pod numbers and seed yield.

CHAPTER 8. GENERAL DISCUSSION AND CONCLUSIONS

Pea is sensitive to heat stress which causes a shortened life cycle, accelerated senescence, ovule, flower and pod abortions, and thus seed yield loss (Lambert and Linck, 1958; Karr et al., 1959; Nonneke et al., 197; Jeuffroy et al., 1990; Pumphrey and Ramig 1990; Guilioni et al., 2003; Sadras et al., 2013; Bueckert et al., 2015). Pea germplasm has genetically diverse morphological and biochemical characteristics which might be involved in heat response (Burstin et al., 2015; Rana et al., 2017). My PhD project was mainly focused on, investigating heat response of diverse pea cultivars, identifying heat tolerance traits from the diverse morpho-anatomical and biochemical characteristics, determining threshold temperatures for various physiological and growth processes, and examining individual and combined effects of heat and drought stresses. Also, I examined effects of plant growth habit, leaf type, canopy color, chlorophyll, carotenoid, anthocyanin and wax on pea heat response and their relationship with leaf spectral reflectance and vegetation indices.

8.1 Overall effects of heat stress on pea physiology, growth and yield

Results from my PhD study have provided detailed heat responses of the diverse pea cultivars grown under different environmental conditions, both in field and controlled conditions. While most pea heat stress studies mainly concentrated on the reproductive phase, my data showed both the vegetative and reproductive phase heat stress reduced plant height, internode length, node formation, pod numbers, pod set ratio and seed yield. The vegetative-phase heat stress causes hormone imbalance and enzyme malfunctioning which contribute to the retarded growth and yield loss (Maestri et al., 2002; Barnabás et al., 2008; Reichler et al., 2009). My data showed concurrent occurrence of heat and drought resulted in the poorest growth and yield both under the controlled and field conditions (Hall, 2004; Bueckert et al., 2015). Pea seed yield is a function of plant population density, pod number per plant, seed number per pod, and weight of individual seeds (French 1990; Jeuffroy et al., 1990; Poggio et al., 2005). Pod set was the trait most affected by heat stress, and compared to controls, 40 and 23% pod reduction was observed under the field and growth chamber conditions respectively. Such reductions were associated with flower and pod abortions due to heat stress, detrimentally impacting pea yield (French 1990; Jeuffroy et al., 1990; Guilioni et al., 1997). Pod set relies on functioning male and female floral components, both of

which are reportedly very sensitive to heat stress (Jiang et al., 2015; Wang et al., 2006). Under a controlled environment, combined occurrence of heat and drought lead to a 57% reduction in pod numbers. Similarly, heat stress lead to a significant reduction in seeds per pod and seed yield, and the loss was exacerbated with drought (Jeuffroy et al., 1990; Guilioni et al., 2003; Daryanto et al., 2015).

Flowering duration was another trait significantly affected by heat or heat and drought combined stresses (Guilioni et al., 2003; Sadras et al., 2012; Bueckert et al., 2015). Flowering duration had a strong negative correlation (r = -0.88***) with air temperature and, and a positive correlation (r = 0.78***) with cumulative rainfall. High temperature induced acceleration of phenology and yield loss was also reported in other pulse crops (Wang et al., 2006; Sita et al., 2017), and cultivars with a capacity to resist heat stress had long flowering phases under the heat stress situation (Berger et al., 2006). Generally, cultivars with vining growth had a longer flowering duration. However it did not result in a yield advantage under the combined heat and drought condition in Saskatoon 2015, likely due to more, flower, pod, and seed abortions (Jeuffroy et al., 1990; Guilioni et al., 2003).

I measured stomatal conductance for the growth chamber experiments under individual and combined heat and drought stresses. While stomatal conductance was significantly reduced due to drought and combined stresses, the impact of heat stress alone was not significant. Reduced stomatal conductance due to stomatal closure is an early plant response to drought stress as an avoidance mechanism by maintaining water in their system (Hsiao 1973; Khan et al., 2010), which was however associated with reduced photosynthesis, growth and yield (Beebe et al, 2013). Stomatal closing under the combined and drought stresses was associated with increased canopy and leaf temperatures mainly due to the inability of plants to cool themselves through transpiration (Alexieva et al., 2001). Canopy temperature is essentially a diagnostic trait and an indicative of the relative fitness of a cultivar to the environment (Jackson et al., 1981). My data showed canopy temperature increased as the weather became hot and dry with high vapor pressure deficit (VPD) such as in the late seeded Rosthern and Saskatoon 2015 trials, and decreased with low air temperature and VPD in normal seeded experiment in Saskatoon 2016. Year 2015 was hot and dry, which increased CT and VPD, respectively by 2 ℃and 0.43 kPa compared with 2016. Canopy temperature was positively correlation with air temperature (r = 0.71***) and the correlation was negative with rainfall (r = 0.81***). The major environmental factors contributing to increased

canopy temperature are the ambient air temperature, excess radiation and high VPD (Havaux, 1989; Will et al., 2013; Siebert et al., 2014). Increased VPD leads to greater transpiration water loss, and thus to drought stresses which may aggravate the heat effect (Will et al., 2013).

Regarding pigment composition and concentrations, results from my PhD project demonstrated a significant loss in chlorophyll a, chlorophyll b, and similar results were reported in other crops (Karim et al., 1999; Alexieva et al., 2001; Dutta et al., 2009; Feng et al., 2014). Such a loss in the chlorophyll concentration arose either due to decreased biosynthesis, chlorophyll degradation, or a combination of both (Karim et al., 1999). Chlorophyll is a key component in light absorption and transfer, and thus the chlorophyll degradation causes impaired photosynthesis and oxidative damage which consequently leads to reduced growth and yield (Berry and Bjorkman, 1980; Guo et al., 2006; Wahid et al., 2007).

Similarly heat stress lead to a decreased carotenoid concentration (Othman et al., 2014). Carotenoid are antenna pigments and have direct influence on the photosynthesis process, and the two major roles are light harvesting during photosynthesis, and minimizing photo-oxidative damage of chlorophyll molecules by dissipating excess energy in the form of heat (Deming-Adam and Adams, 1996; Misra et al., 2006). Contrary to chlorophyll and carotenoid, heat stress lead to an increased petiole and leaf surface anthocyanin concentrations. Stressful environment trigger the formation of harmful reactive oxygen species (ROS), and free radicals (Tripathy and Oelmuller, 2012). To protect plants from the harmful effects of ROS, high level of antioxidants is needed, and anthocyanins were reported to fulfill such a role of ant-oxidant effects in several crops (Barker et al., 1997; Hatier and Gould, 2008). Compared to the non-heat stressed environment, the heat stressed environment had 29.6% higher total leaf wax concentration. Moreover, during the reproductive stage wax concentration increased by 47.8 and 44.1% in heat stressed and non-stressed environments, respectively.

8.2 Threshold temperature for various pea growth processes

Threshold air temperatures for heat stress lack consistency in the literature ranging from 15-32 °C under field conditions (Lambert and Lick, 1958; Stanfield et al., 1966; Nonnecke et al, 1971; Pumphrey and Ramig, 1990; Sadras et al., 2012; Bueckert et al., 2015; 2015) and 31-37 °C under controlled growth chambers (Jeuffroy et al., 1990; Jiang et al., 2015). Such a huge variation in the threshold temperate suggested that, in addition to the air temperature other environmental, growth stage and genetic factors might have contributed to the threshold temperature. My data

showed various plant processes have different threshold temperatures. As air temperature increased, there was an immediate response in stomatal conductance and leaf temperature. Significant chlorophyll and carotenoid concentrations reduction was observed after 7-day heat exposure at 31 °C and beyond whereas the corresponding wax and anthocyanin concentrations was significantly increased at 28 °C and beyond. Plant height and node formation were not affected until the temperature reached 34 °C in controlled environment conditions whereas pod numbers, seed numbers per pod, and seed yield had lower (≤ 31 ºC) threshold temperatures. Apart from heat intensity, the duration of exposure had a great impact on pea heat response. The longer duration of heat exposure led to more damage and the difference between the control and the heat regimes increased with the number of days under the heat treatments. Marias et al (2017) showed longer heat exposure led to greater physiological damage and slower recovery in coffee seedlings. Prasad et al., (2017) indicated long exposures reduced harvest index of several field crops including wheat and legume crops. My results revealed heat stress during late vegetative stage significantly reduced pea growth and yield and the damage increased with both the intensity and duration of heat.

Regarding leaf development stage, my data showed expanding leaves are more sensitive to heat stress than expanded and senescing leaves, which is in agreement with a report on coffee seedlings where expanding leaves were more sensitive to heat stress, with decreased photosynthesis and less recovery from heat (Marias et al., 2017). The heat sensitivity of the expanding leaves could also be associated with the lesser wax concentration. Cuticle wax concentration of leaves is inversely related to leaf temperature, so leaves with more wax can reflect more heat as presented in chapter 7, and thus reduce the leaf temperature (Sánchez et al., 2001; Jones and Rotenberg 2011).

8.3 Morphological traits for heat resistance

Under the most stressed environment cultivars with upright canopy habit and semileafless leaf, had a high heat tolerance index, low canopy temperature, high pod number and a high pod set ratio, which suggested the upright and semileafless nature contributed to heat avoidance (Alvino and Leone, 1993; Baigorri et al., 1999). Cultivars with an upright canopy habit avoided direct contact with the hot soil surface, and the upright canopy allowed enhanced aeration within the canopy, to contribute to a cooler canopy temperature on hot days (Alvino and Leone, 1993; Heath and Hebblethwaite, 1985). An upright canopy is more erectophile, so light radiation is reflected off surfaces to a greater extent (Heath and Hebblethwaite 1987). The semileafless leaf

has smaller lamina with more leaf stalks (tendrils and petiole) all of which likely help conserve water by decreasing transpiration loss (Reicosky et al., 1980; Wilson et al., 1981; Kashiwagi et al., 2008), and thus to a cooler canopy temperature than the normal leaf. In a study conducted under moisture stress, semileafless pea cultivars used water more efficiently under drier conditions, with an advantage of photosynthetic contribution from tendrils (Wilson et al., 1981). Moreover, semileafless cultivars often have better resistance against disease and pest attacks (Snoad 1974), which usually contribute to higher canopy temperature. These features and qualities make semileafless pea cultivars more tolerant to heat stress over cultivars with the normal leaf. In contrast, under the non-stressed condition, cultivars with a normal leaf, an indeterminate growth habit with vining canopy, had greater performance in yield and growth-related traits, and a similar result was observed in chickpea and its ecotypes (Berger et al., 2006).

A strong positive correlation ($r = 0.73$, $P < 0.01$) was observed between lodging and canopy temperature, demonstrating that lodging exacerbated heat stress on the crop (Alvino and Leone, 1993). Under field conditions, lodged cultivars make direct contact with the hot soil surface, from which heat can transfer to the plant canopy as conducted heat (Flerchinger et al., 2003). Lodged canopies are planophile, and absorb more heat from the sun and reflect less, and this further results in a hotter canopy. On hot days, when air temperature > 27 °C, the soil surface temperature was roughly 15 °C higher than canopy temperature (our unpublished data).

From WBI measurement, pea cultivars with the normal leaf had lower leaf water content than the cultivars with semileafless leaf. In water deficit stress, heat stress was aggravated by the inability of the plant to effectively reduce canopy temperature through transpirational cooling (Kashiwagi et al., 2008). Based on results of a heat and moisture stress study on soybean, (Reicosky et al., 1980) concluded that high radiation and moisture stress contributed to increased canopy temperature on hot days.

8.4 Pigments and waxes as heat resistance traits

Heat stress decreased chlorophyll concentrations, which ultimately reduces the plants photosynthetic activity and induce oxidative damage, and then leads to yield loss (Berry and Bjorkman, 1980; Guo et al., 2006; Wahid et al., 2007; Bita and Gerats, 2013). Interestingly, my data showed an increased chlorophyll a/b ratio under the heat stressed environment, likely due to a faster chlorophyll b degradation than chlorophyll a, indicating a differential sensitivity in light-harvesting chlorophyll a/b-binding proteins complex (Plumley al., 1995). Although, a change in

chlorophyll a/b ratio associates in the plant's heat response (Cui et al., 2006), reports were inconsistent on how the chlorophyll a/b ratio is linked with the heat response. Some reports indicated an increased chlorophyll a/b ratio as an indicator of heat tolerance (Cui et al., 2006; Wahid, 2007; Bita and Gerats, 2013), while others showed the opposite (Feng et al., 2014). My data showed cultivars with high (> 3.3) chlorophyll a/b ratio including CDC Meadow and Naparynk also had high heat tolerance index, suggesting high chlorophyll a/b ratio likely associates with heat tolerance (Cui et al., 2006). The chlorophyll a/b ratio shows the relative sensitivity of the light harvesting complex and the reaction center (Cui et al., 2006). Although the optimal chlorophyll a/b ratio needs further study, both too high and too low chlorophyll a/b ratios suggest damage at the antenna complex or the reaction center, respectively (Guo et al., 2006; Feng et al., 2014).

Carotenoid are antenna pigments and have direct influence on the photosynthesis process, and the two major roles are light harvesting during photosynthesis, and minimizing photo-oxidative damage of chlorophyll molecules by dissipating excess energy in the form of heat (Deming-Adam and Adams, 1996; Misra et al., 2006). Carotenoid concentration had positive association with heat tolerance index, and contributed to low canopy temperature. Carotenoid concentration negatively correlated with canopy temperature and the correlation was positive with the heat tolerance index. Higher concentration of anthocyanin was associated with the most heat stressed environment. In Brassica crops, anthocyanin production was enhanced in response to most environmental stresses including heat, drought, and light (Shepherd and Griffiths, 2006). However, stressful environments also trigger the formation of harmful reactive oxygen species (ROS), and free radicals (Tripathy and Oelmuller, 2012). To protect plants from the harmful effects of ROS, high level of anti-oxidants is needed, and anthocyanins were reported to fulfill such a role in several crops (Barker et al., 1997; Hatier and Gould, 2008). Anthocyanin protect chloroplast by reducing incident light on it, and have antioxidant role through scavenging reactive oxygen species (Yamasaki et al., 1996; Yamasaki, 1997). Also, anthocyanins protect sensitive plant tissues by screening damaging UV radiation (Barker et al., 1997; Singh et al., 1999), and their concentration increases in response to heat stress (Hosseinian et al., 2008)

While roles of epicuticular wax as a drought tolerance trait was extensively reported over a range of crops (Shepherd and Griffiths, 2006; Sanchez et al., 2001; Ebrcon et al., 1977; Guo et al., 2016; Willick et al., 2017), relatively little has been reported on its role as a heat stress tolerance

trait. My result showed a significant variation among pea cultivars in both lamina and petiole bulk wax concentration under heat stressed, and non-stressed environments. Wax composition and concentration variability has also been reported within other pea cultivars, and between several crop species (Ebercon et al., 1977, Sanchez et al., 2001). Compared to the non-heat stressed environment, heat stressed environment had 29.6% higher total leaf wax concentration. Moreover, during the reproductive stage wax concentration increased by 47.8 and 44.1% in heat stressed and non-stressed environments, respectively. These results indicated, genetic factor (cultivar), plant age and heat stress contributed to the leaf wax biosynthesis, but environment was the most determinant factor (Shepherd and Griffiths, 2006).

In regard to heat avoidance, epicuticular wax has two major roles, protecting from excess heat or radiation by reflecting ultraviolet, visible and infrared wavelengths (Jefferson et al., 1989; Shepherd and Griffiths, 2006), and also stay minimizing water loss through reduced residual transpiration (Sanchez et al., 2001; Guo et al., 2016). Previous studies on pea and other crops have associated epicuticular wax with improved drought tolerance (Sanchez et al., 2001; Kosma et al., 2009; Zhang et al., 2015), and wax reduces night time water loss.

Generally high wax concentration under heat stress was associated with a decreased canopy temperature, and a higher heat tolerance index. Cultivars with darker or bluish-green leaves had higher lamina wax concentration than the light green leafed cultivars. Cultivars with an upright growth habit and the semileaf leaf, both stress hardy traits, were also associated with higher wax concentration under stressed environments. Wax accumulation positively associated with water band index, a proxy for leaf water content, indicating that leaf surface wax was minimizing water loss. Generally, glaucousness or waxy leaves helped to maintain high water potential in other crops like wheat and can therefore be considered as a trait for drought tolerance (Richards et al., 1986; Ludlow and Muchow, 1990). Glaucousness can be considered as a trait for heat tolerance, because indirectly I noted sufficient water supply moderated heat stress in chapter 6. Richards et al (1986) indicated a 0.7 ℃ decrease in leaf temperature between waxy and non-waxy wheat cultivars.

Epicuticular waxes enhance leaves radiation reflectance capability and thus protect excess radiation and heat associated damage (Grant et al., 1995). In contrast, the wax removal (dewax), lead to a decreased reflectance percentage both in the UV and NIR regions suggesting loss of protective cover from the heat and radiation damages (Holmes and Keiller 2002). The exogenous

wax application significantly decreased stomatal conductance which has been reported as a strategy to minimize water loss (Sánchez et al., 2001).

8.5 Spectral reflectance and vegetation indices association with heat stress

Radiation and heat reflectance has been reported as a heat avoidance strategy during hot days (Peñuelas et al., 2004; Cossani and Reynolds 2012), which was reportedly associated with leaf surface properties such as wax and pigment composition and concentrations (Shepherd and Griffiths 2006). My results are in agreement with other studies which reported a positive association between epicuticular wax concentration and reflectance percentage both in the UV and NIR regions (Holmes and Keiller 2002; Grant et al., 1995).

The greater spectral reflectance in the UV region protects the leaf from the high energy UV damage (Holmes and Keiller 2002). Similarly, the greater reflectance in the NIR regions decreases heat load and associates with vigor and overall plant health (Babar et al., 2006).

Vegetative indices (VIs) determined from the spectral reflectance indicate the overall physiological status and the plants stress tolerance level (Gamon et al., 1997; Xue and Su 2017). Generally, three main categories of traits can be estimated from the different VIs based on the reflectance wavelengths (Xue and Su 2017). The first group VIs include those indices derived from the visible spectral region, including photochemical reflectance index (PRI), normalized pigment and vegetation index, and carotenoid reflectance index (Gamon et al., 1997; Peñuelas et al., 2004). The shade treatment had the most obvious impact on the visible spectral region thus the indices determined from the shade treatment significantly varied from the added wax and untreated control. For example, the PRI significantly reduced by the shade treatment. Photochemical reflectance index is a direct indicator of the plant's photosynthetic radiation use efficiency (Gamon et al., 1997; Babar et al., 2006). Interestingly, my result also showed a significant correlation between the PRI chlorophyll concentrations. Researches also showed significant association between PRI net CO_2 uptake and radiation use efficiency (Gamon et al., 1997).

The second group involves reflectance in the visible and near infrared regions from which VIs that indicate vegetation vigor, greenness, and rate of senescence (Babar et al., 2006). The most common VIs of this group includes NDVI and its derivatives. The shade treatment that led to chlorophyll loss, significantly reduced NDVI in all pea cultivars. In a different study, I reported that heat stress leads to chlorophyll degradation in pea, and several studies reported that stay green

under environmental stress is a trait directly associates with stress tolerance (Cossani and Reynolds 2012). The NDVI along with other VIs can thus indicate the crops' stress tolerance level.

The third group involves vegetation indices derived from the near infrared region reflectance, which are proxies mainly for the tissue water status (Zarate-Valdez et al., 2012). The typical index in this group is water band index band index (WBI) (Penuelas et al., 1997). My result showed a significant negative correlation between WBI and canopy temperature, and positive association between WBI and wax concentration.

8.6 Sufficient water supply increases threshold temperature and minimizes heat effects

In contrary, heat stress with availability of sufficient water was associated with a cooler LT as evidenced by a 2.2 ℃ reduction compared to the combined stress. Interestingly, pea treated under combined and drought stress showed similar pattern of responses (Grigorova et al., 2011; Awasthi et al., 2014) in g_s and ET though the combined stress lead to a very high LT. I also noticed that pea subjected to drought stress had 1 ℃high LT than the control, which suggested that drought could lead to heat stress. Such an increase of LT was due to stomatal closure and the inability of plants to cool themselves through transpiration cooling (Khan et al., 2010). Heat stress almost always had lower effect on pea growth parameters than drought or combined stresses, indicating heat stress was moderated by the availability moisture which enhanced transpiration cooling (Figure 7.5E). During the treatment duration, the LT increase of peas under the control and heat treatments was low compared to the corresponding LT increase of peas under drought and combined stresses, suggesting the negative impacts of heat stress due to high air temperature can be mitigated by an optimal supply of water. In all yield parameters, the effect of heat stress alone was relatively milder than drought and combined stress which suggests the maximum threshold temperature affecting pea performance regarding seed yield and its components such as number of pods per plant, pod set ratio, and single seed weight can be increased if moisture is not a limiting factor. Under high temperature condition, availability of optimal moisture enhanced yield in a controlled environment. Also, under field conditions Bueckert et al (2015) indicated that precipitation increased pea yield in hot years. Such results strongly suggest the possibility of mitigating the influence heat stress by optimal irrigation.

8.7 Conclusions

Pea is sensitive to both vegetative and reproductive stage heat stresses, although the threshold temperature for the vegetative stage is high compared with the reproductive stage. Pea cultivars varied in heat responses, susceptible to tolerant. Canopy and leaf temperatures indicate the level of heat stress and the crop physiological status. Leaf and canopy traits played key roles in the heat avoidance and tolerance. Under heat stressed environments, cultivars with the semileafless leaf, determinate growth habit, and upright canopy habit, maintained a cooler canopy and demonstrated improved yield including pod number and pod set ratio. Lodging exacerbated heat susceptibility. In contrast, under optimal environments, the normal leaf type, indeterminate and vining canopy habit had a greater potential for growth-related traits and more pods in the growing season.

In this study I noticed two major roles of leaf surface waxes as a heat avoidance trait: 1) reducing radiation and heat load by enhanced reflectance both in the high energy ultraviolet radiation and in the near infrared regions, and 2) minimizing water loss due to the decreased stomatal conductance and higher plant water content. For the first time, I demonstrated the possibility of exogenous wax application on leaf surfaces to augment the naturally existing wax content and enhance the plants' heat avoidance capacity. Chlorophyll a, chlorophyll b and carotenoid concentrations decreased due to heat stress whereas anthocyanin and wax concentrations increased. A relatively low (< 3.1) chlorophyll a/b ratio indicates heat tolerance. Shading led to chlorophyll and carotenoid loss. Leaf spectral reflectance highly dependent on pigments, wax, and water content. Vegetative indices determined from spectral reflectance measurement can be used as proxies for pigments, wax, and water contents. The VIs are able to indicate the overall physiological and biochemical status of a plant without involving costly and time consuming laboratory procedures.

Pea threshold temperature is dependent on cultivar, phenology, and there is specific threshold temperature for the different physiological and growth processes. For example, plant height and node formation have high threshold (> 34 ºC) but pod number and yield related traits were affected at 31 ºC. Chlorophyll and carotenoid concentration decreased at (34 ºC), whereas wax and anthocyanin increased from 24 to 34 ºC, and then started to decline beyond that. Pea plants grown under the controlled environment (growth chamber) have 5-7 ºC higher threshold temperature than the field conditions. If water is not limiting, stomatal conductance increases with

heat stress, until certain level. Duration of heat exposure was equally important as the intensity of the heat and the longer the duration, the more the negative effect. Yield traits with the lowest (≤ 31 °C) thresholds should be targeted first in breeding programs to improve pea adaptation to warmer climates. Expanding leaves were more sensitive to heat stress and had a lower threshold temperature than mature and senescing leaves. Expanding and senescing leaves had greater anthocyanin concentration than matured leaves.

Heat and drought stress have both common and unique effects on pea growth, physiology and yield. Stomatal conductance and ET decreased due to drought and combined stresses, however these traits were either unaffected or slightly increased due to heat stress. Although all stresses increased leaf temperature, abundant soil moisture enabled the heat treatment to decrease leaf temperature by 2.2 ºC compared with pea under the combined stress. Evapotranspiration and stomatal conductance were associated with lower leaf temperature under heat stress. Seed yield was strongly associated with pod numbers and total seed numbers but the association with seed size was low. Overall, concurrent drought and heat had the most detrimental impact on pea growth and yield followed by the drought stress. An optimal soil water supply can therefore moderate the impacts of heat stress.

Pea cultivars have various degree of tolerance to the different stress regimes. Generally, CDC Meadow was heat tolerant, and moderately tolerant to drought, but sensitive to the combined stresses. CDC Golden was moderately tolerant to all stress conditions. CDC Sage and Cooper were sensitive to environmental stresses from the four cultivars used in this study.

8.8 Future Research

This thesis provides substantial information to advance my knowledge of improving pea heat tolerance based on canopy and leaf traits. I have identified major traits involved in pea heat avoidance and tolerance and highlighted management aspects for heat avoidance. However, there are several areas and gaps that need to be addressed in future research:

1. Semileafless and upright traits associated with heat tolerance index, low canopy temperature and high yield potential under heat stressed environment. In contrast, vining and normal leaf cultivars had high yield potential under non-stressed conations but not in the heat stressed condition. A breeder may be interested in reconciling heat tolerance with yield potential to generate high yielding cultivars with heat tolerance trait.

2. I have identified role of wax and pigments as heat tolerance traits. High wax concentration associates with high spectral reflectance both in the ultraviolet and near infrared regions which intern associates with heat and excess radiation avoidance. However, the wax trait presented in this thesis was only the bulk wax, the wax composition under varying condition needs further study.
3. I have identified vegetation indices including NDVI, PRI, NPCI, WBI, ARI, CRI, and GNDVI which be used as proxies for heat tolerance and their association with pigment, wax and water content, and thus with heat avoidance. I only studied leaf reflectance with limited pigment and wax treatment manipulation. The transmittance and absorption components warrant further study. Moreover, the role of cellular components including palisade mesophyll cells, and cytoplasmic organelles need further study. The vegetative indices associated with heat tolerance may be of interest to breeders or molecular biologist to search for genes, and the proteins associated these putative genes.
4. I highlighted that high canopy and leaf temperature is an indicator of heat or water-deficit stress. It can also indicate development stage (phenology), because I have noticed an increasing trend in leaf temperature with plant development stage. Similarly, pigment concentration may be varied due to both heat and tissue development (age). The heat stress association with plant and tissue development needs further study.
5. In heat stress experiments, in addition to a genetic aspect environmental factors are the primary factors. The radiation balance, even a full energy balance, needs to be addressed.
6. Heat resistance is associated with water availability, stomatal conductance, cuticular transpiration, residual transpiration, and these processes are directly related to root function. Roots (‘the hidden half’) and their association with heat stress is neglected and warrants research
7. As a management option to avoid heat stress, I demonstrated the possibility of moderating heat stress by sufficient water supply and by exogenous wax application. Also, use of a normal seeding time helped to avoid heat during flowering. These aspects require further study in designed field experiments.
8. I started role of heat acclimation and preconditioning on pea heat avoidance, and saw promising results (data not shown in the thesis). It needs further study.

9. My study focused on leaf and canopy aspects, growth and phenology. More research is needed on molecular, protein, and cellular based tolerance. Also, approaches from genomics, proteomics and transcriptomic areas will reveal a more detailed understanding of the molecular basis of the plant heat response.

REFERENCES

Abdel-Aal, E. S.M., and Hucl, P. 1999. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chem. 76:350–354.

Alexieva, V., Sergiev, I., Mapelli, S., and Karanov, E. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 24: 1337–1344. doi:10.1046/j.1365-3040.2001.00778.x.

Alonso-Blanco, C., Aart, M.G., Bentsink, L., Keurentjes, J.J., Reymond, M., Verugdenhil, D., and Koomneef, M. 2009. What has natural variation taught us about plant development physiology and adaptation? Plant Cell 21:1877–1896.

Alvino, A., and Leone, A. 1993. Response to low soil water potential in pea genotypes (*Pisum sativum* L.) with different leaf morphology. Scientia Hort. 53: 21–34.

Amani, I., Fischer, R.A., Reynolds, M.P. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. J. Agron. Crop Sci. 176:119–129.

Aparicio, N., Villegas, D., Casadesus, J., Araus, JL., and Royo, C. 2000. Spectral vegetation indices as non-destructive tools for determining durum wheat yield. Agron. J. 92: 83–91

Awasthi, R., Kaushal, N., Vadez, V., Turner, N.C., Berger, J., Siddique, K.H.M., and Nayyar, H. 2014. Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. Funct. Plant Biol. 41: 1148–1167. doi:10.1071/FP13340.

Babar, M.A., Reynolds, M.P., Ginkel, M. Van, Klatt, A.R., Raun, W.R., and Stone, M.L. 2006. Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. Crop Sci. 46:1046–1057 doi:10.2135/cropsci2005.0211.

Baigorri, H., Antolı´n, M.C., and Sa´nchez-Dı´az, M. 1999. Reproductive response of two morphologically different pea cultivars to drought. Eur. J. Agron. 10:119–128.

Baldev, B. 1988. Origin, distribution, taxonomy, and morphology. In Pulse Crops. pp. 3–51. B.

Balota, M., Payne, W.A., Evet, S.R., Lazar, M.D. 2007. Canopy temperature depression sampling to assess grain yield and genotypic differentiation in winter wheat. Crop Sci. 47: 1518–1529.

Barker, D.H., Seaton, G.G.R., and Robinson, S.A. 1997. Internal and external photo-protection in developing leaves of the CAM plant Cotyledon orbiculata. Plant Cell Environ. 20: 617–624.

Barnabas, B., Jäger, K., and Fehér, A. 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 31: 11–38.

Battisti, D., Naylor, R.L. 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323: 240–244.

Bastianelli, D., Grosjean, F., Peyronnet, C., Duparque, M., and Regnier, J.M. 1998. Feeding value of pea (*Pisum sativum*, L.) chemical composition of different categories of pea. Animal Sci. 67: 609–619.

Beebe, S.E., Rao, I.M., Blair, M.W., and Acosta-Gallegos, J.A. 2013. Phenotyping common beans for adaptation to drought. Front. Physiol. 4: 1–20. doi:10.3389/fphys.2013.00035.

Berger, J.D., Ali, M., Basu, P.S., Chaudhary, B.D., Chaturvedi, S.K., Deshmukh, P.S., Dharmaraj, P.S., Dwivedi, S.K., Gangadhar, G.C., Gaur, P.M., Kumar, J., Pannu, R.K., Siddique, K.H.M., Singh, D.N., Singh, D.P., Singh, S.J., Turner, N.C., Yadava, H.S., and Yadav, S.S. 2006. Genotype by environment studies demonstrate the critical role of phenology in adaptation of chickpea (*Cicer arietinum* L.) to high and low yielding environments of India. Field Crops Res. 98: 230–244. doi:10.1016/j.fcr.2006.02.007.

Berry, J. A., and Bjorkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol.31:491–543.

Broge, N.H., Leblanc, E. 2001. Comparing prediction power and stability of broadband and hyperspectral vegetation indices for estimation of green leaf area index and canopy chlorophyll density. Remote Sens. Environ. 76: 156–172.

Bueckert, R.A., and Clarke, J.M. 2013. Review: Annual crop adaptation to abiotic stress on the Canadian prairies: six case studies. Can. J. Plant Sci. 93: 375–385. doi:10.4141/cjps2012-184.

Bueckert, R.A., Wagenhoffer, S., Hnatowich, G., and Warkentin, T.D. 2015. Effect of heat and precipitation on pea yield and reproductive performance in the field. Can. J. Plant Sci. 95: 629–639. doi:10.4141/cjpS2014-342.

Buschhaus, C., and Jetter, R. 2011. Composition differences between epicuticular and intracuticular wax substructures: how do plants seal their epidermal surfaces. J Exp. Bot. 62: 841–85.

Carter, G.A, 1998. Reflectance wavebands and indices for remote estimation of photosynthesis and stomatal conductance in pine canopies. Remote Sens. Environ.63: 61–72.

Cazzonelli, C. I., and Pogson, B. J. 2010. Source to sink: regulation of carotenoid biosynthesis in plants. Trends Plant Sci. 15: 266–274. doi: 10.1016/j.tplants.2010.02.003.

Ceccarelli, S. 1987. Tolerance to climatic stress. Barley Genetics V. Proceedings of 5th Int. Barley Genetics Symposium, Okyama, pp. 689-702.

Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70:1–9.

Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70: 1–9.

Chappelle, E.W., Kim, M.S., and McMurtrey, J.E. 1992. Ratio analysis of reflectance spectra

(RARS): An algorithm for the remote estimation of the concentrations of chlorophyll A, chlorophyll B, and carotenoid in soybean leaves. Remote Sens. Environ. 39: 239–247.

Chen, T.H.H., Shen, Z.Y., and Lee, P.H. 1982. Adaptability of crop plants to high temperature stress. Crop Sci. 22: 719–725.

Cousin, R. 1997. Peas (*Pisum satvum* L.). Field Crop Res. 53: 111–130.

Cossani, C.M., and Reynolds, M.P. 2012. Physiological traits for improving heat tolerance in wheat. Plant Physiol. 160: 1710–1718. doi:10.1104/pp.112.207753.

Cote, R., Gerrath, J.M., Posluszny, U., and Grodzinski, B. 1992. Comparative leaf development of conventional and semileafless peas (*Pisum sativum* L.) Can. J. Bot. 70: 571–580.

Creelman, R. A., Tierney, M. L., and Mullet, J. E. 1992. Jasmonic acid/methyl jasmonate accumulation in wounded soybean hypocotyls and modulate wound gene expression. Proceedings of the National Academy of Science 89: 4938-4941.

Cui, L., Li, J., Fan, Y., Xu, S., and Zhang, Z. 2006. High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. Botanical Studies 47: 61–69.

Cutforth, H., and Judiesch, D. 2012. Temperature trends in the semiarid prairie of southwestern Saskatchewan revisited. Can. J. Soil Sci. 92: 803–806. doi:10.4141/cjss2012-015.

Cure, W.W., Flagler, R.B., and Heagle, A.S. 1989. Correlations between canopy reflectance and leaf temperature in irrigated and droughted soybeans. Remote Sens. Environ. 29: 273–280. doi:10.1016/0034-4257(89)90006-0.

Dahl, W. J. Foster, L. M.–Tyler, R. T. 2012. Review of the health benefits of peas (*Pisum sativum* L.). Br. J. Nutr. 108: 3–10. doi: 0.1017/S0007114512000852.

Daryanto, S., Wang, L., and Jacinthe, P.A. 2015. Global synthesis of drought effects on food legume production. PLoS One 10: 1–16. doi:10.1371/journal.pone.0127401.

Davies, D.R. 1977. Restructuring the pea plant. Science Progress 64: 201–214.

De Boeck, H. J., Van De Velde, H., De Groote, T., and Nijs, I. 2016. Ideas and perspectives: heat stress: more than hot air. Biogeosci. 13: 5821–5825. doi:10.5194/bg-13-5821-2016.

Demmig-Adams, B., and Adams, W.W. 1996. The role of xanthophyll cycle carotenoid in the protection of photosynthesis. Trends in Plant Sci. 1: 21–26. doi:10.1016/S1360-1385(96)80019-7.

Dixon, R. A., Harrison, M. J., and Lamb, C. J. 1994. Early events in the activation of plant defence responses. Annu. Rev. Phytopathol. 32: 479-501.

Dutta, S., Mohanty, S., and Tripathy, B.C. 2009. Role of temperature stress on chloroplast biogenesis and protein import in pea. Plant Physiol. 150: 1050–1061. doi:10.1104/pp.109.137265.

Ebercon, A., Blum, A., Jordan, W.R. 1977. A rapid colorimetric method for epicuticular wax content of sorghum leaves. Crop Sci. 17: 179–180.

Ehlers, J.D., Hall, A.E. 1998. Heat tolerance of contrasting cowpea lines in short and long days. Field Crops Res. 55: 11–21.

El-Hendawy SE, Hassan WM, Al-Suhaibani NA, Schmidhalter U. 2017. Spectral assessment of drought tolerance indices and grain yield in advanced spring wheat lines grown under full and limited water irrigation. Agric. Water Management. 182:1–12. https://doi.org/10.1016/j.agwat.2016.12.003

Espósito, M.A., Martin, E.A., Cravero, V.P., Liberatti, D., López Anido, F.S., and Cointry, E.L. 2009. Relationships among agronomic traits and seed yield in pea. J. Basic Appl. Genet. 20: 1–8.

FAOSTAT, 2016 http://www.fao.org/faostat/en/#data/QC. Accessed on July 17, 2018.

Farroq, M., Siddique, K.H.M., Varshney, R.K., and Nayyar, H. 2017. Food legumes and rising temperatures: effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. Front. Plant Sci. 8:1658. doi: 10.3389/fpls.2017.01658

Feller, U., Crafts-Brander, S.J., Salvucci, M.E. 1998. Moderately high temperatures inhibit ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. Plant. Physiol. 116:539–546.

Feng, B., P. Liu, G. Li, S. T. Dong, F. H. Wang, L. A. Kong, and Zhan, J.W. 2014. Effect of heat stress on the photosynthetic characteristics in flag leaves at the grain-filling stage of different heat-resistant winter wheat varieties. J. Agron. Crop Sci. 200: 143–155.

Fernandez, G.C.J. 1992. Effective selection criteria for assessing stress tolerance. In: Kuo, C.G. (Ed.), Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress, Publication, Tainan, Taiwan.

Ferreres, F., Gil, M. I., Castaner, M., and Thomas-Barberan, F. A. 1997. Phenolic metabolites in red pigmented lettuce (*Lactuca sativa* L.). Changes with minimal processing and cold storage. Journal of Agriculture and Food Chemistry 45: 4249-4254.

Filella, I., Serrano, L., Serra, J., and Peñuelas, J. 1995. Evaluation of wheat crop nitrogen status by remote sensing: reflectance indices and discriminant analysis. Crop Sci. 35: 1400–1405.

Fischer, R., and Maurer, R. 1978. Drought resistance in spring wheat cultivars, 1.Grain yield responses. Aust. J. Agric. Res. 29: 897. doi:10.1071/AR9780897.

Flerchinger, G.N., Sauerb, T.J., and Aikenc, R.A. 2003. Effects of crop residue cover and architecture on heat and water transfer at the soil surface. Geoderma 116:217–233. doi:10.1016/S0016-7061(03)00102-2.

Fleury, D., Jefferies, S., Kuchel, H., Langridge, P. 2010. Genetic and genomic tools to improve

drought tolerance in wheat. J. of Exp. Botany 61: 3211–3222.

French, R.J. 1990. The contribution of pod numbers to field pea (*Pisum sativum* L.) yields in a short growing-season environment. Aust. J. Agric. Res. 41: 853–862. doi:10.1071/AR9900853.

Fukuoka, N., Suzuki, T., Minamide, K., and Hamada, T. 2014. Effect of shading on anthocyanin and non-flavonoid polyphenol biosynthesis of *Gynura bicolor* leaves in midsummer. Hort. Science 49:1148–1153.

Gamon, J.A., and Surfus, J.S.1999. Assessing leaf pigment content and activity with a reflectometer. New Phytol. 143:105–116.

Gamon, J.A., Serrano, L., and Surfus, J.S. 1997. The photochemical reflectance index: An optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. Oecologia 112: 492–501. doi:10.1007/s004420050337.

Gitelson, A., and Merzlyak, M.N. 1994. Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum* L., and *Acer platanoides* L. leaves spectral features and relation to chlorophyll estimation. J. of Plant Phys. 143: 286–292. doi:10.1016/S0176-1617(11)81633-0.

Gitelson, A. A., Merzlyak, M.N., and Chivkunova, O.B. 2001. Optical properties and non-destructive estimation of anthocyanin content in plant leaves. Photochem. Photobiol. 74: 38–45.

Gitelson, A. A., R. Stark, U. Grits, D. Rundquist, Y. Kaufman, and Derry, D. 2002. Vegetation and soil lines in visible spectral space: A concept and technique for remote estimation of vegetation fraction. Int.J of Remote Sensing 23: 2537–2562.

Gniwotta,F., Vogg, G., Gartmann, V., Carver, T.L.W., Riederer, M., and Jetter, R. 2005. What do microbes encounter at the plant surface? Chemical composition of pea leaf cuticular waxes1. Plant Physiol. 139: 519–530.

Goldman, L.I., Gritton, T.E., and Flannery, J.P. 1992. Evaluation of the afila-tendriled (*afaf-tactac*) pea foliage type under high competition. Crop Sci. 32: 855–861.

Gonzalez, R., Paul, N.D., Percy, K. Ambrose, M., McLaughlin, C.K., Barnes, J.D., Areses, M., and Wellburn, A.R. 1996. Responses to ultraviolet-B radiation (280-315 nm) of pea (*Pisum sativum* L.) lines differing in leaf surface wax. Physiol. Plant 95: 852–860.

Goodwin, M. 2008. Crop profile for field pea in Canada. Pesticide Risk Reduction Program, Pest Management Centre, and Agriculture and Agri-Food Canada. http://publications.gc.ca/collections/collection_2009/agr/A118-10-3-2009E.pdf (accessed 7 Jan. 2016).

Gourlay, C.W., Hofer, J.M., and Ellis, T.H. 2000. Pea compound leaf architecture is regulated by interactions among the genes UNIFOLIATA, cochleata, *afila*, and tendril-lessn. Plant Cell

12: 1279–1294. doi:10.1105/tpc.12.8.1279.

Grant, R.H., Jenks, M.A., Rich, P.J., Peters, P.J., and Ashworth, E.N. 1995. Scattering of ultraviolet and photosynthetically active radiation by S*orghum bicolor*: influence of epicuticular wax. Agric. For. Meteorol. 75: 263–281. doi:10.1016/0168-1923(94)02210-B.

Grigorova, B., Demirevska, K., and Feller, U. 2011. Combined drought and heat stress in wheat: changes in some heat shock proteins. Biol. Plantarum. 55: 105–111. DOI: 10.1007/s10535-011-0014-x.

Gritton, E.T. 1980. Field pea. In: W.R. Fehr and H.H. Hadley, editors, Hybridization of crop plants. ASA and CSSA, Madison, WI. p. 347–356. doi:10.2135/1980.hybridizationofcrops.c23

Guilioni, L., Wéry, J., and Lecoeur, J. 2003. High temperature and water deficit may reduce seed number in field pea purely by decrease in plant growth rate. Func. Pl. Biol. 30: 1151–1164.

Guilioni, L., Wery, J., and Tardieu, F. 1997. Heat stress induced abortion of buds and flowers in pea: is sensitivity linked to organ age or to relations between reproductive organs? Ann. Bot. 80: 159–168. doi:10.1006/anbo.1997.0425.

Guo, X. R., Cao, K. F., and Xu, Z. F. 2006. Acclimation to irradiance in seedlings of three tropical rain forest *Garcinia* species after simulated gap formation. Photosynthetica 44:193–201.

Hall, A.E. 1992. Breeding for heat tolerance. Plant Breed. Rev. 10, 129–168.

Hall, A. E. 2001. Crop responses to environment. Boca Raton: CRC Press.

Hall, A. E. 2004. Breeding for adaptation to drought and heat in cowpea. Eur. J. Agron. 21: 447–454.

Hasanuzzaman, M., Nahar, K., Alam, M.M., Roychowdhury, R., and Fujita, M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int. J. Mol. Sci. 14: 9643–9684. doi:10.3390/ijms14059643.

Hasanuzzaman M, Davies, N.W, Shabala, L., Zhou, M., Brodribb, T.J., and Shabala, S. 2017. Residual transpiration as a component of salinity stress tolerance mechanism: a case study for barley. BMC Plant Biol. 17: 107. doi 10.1186/s12870-017-1054-y.

Hasegawa, H., Fukasawa-Akada, T., Okuno, T., Niizeki, M., and Suzuki, M. 2001.Anthocyanin accumulation and related gene expression in Japanese parsley (*Oenanthe stolonifera* DC) induced by low temperature. Journal of Plant Physiology 158: 71-78.

Hatfield, J.L., Boote, K.J., Kimball, B.A., Ziska, L.H., Izaurralde, R.C., Ort, D., Thomson, A.M., and Wolfe, D. 2011. Climate impacts on agriculture: Implications for crop production. Agron. J. 103: 351–370. doi:10.2134/agronj2010.0303.

Hatier, J.H., Gould, K.S. 2008. Foliar anthocyanins as modulators of stress signals. J Theor Biol

253:625–627.

Havaux, M. 1989. Increased thermal deactivation of excited pigments in pea leaves subjected to photoinhibitory treatments. Plant Physiol. 89: 286–292.

Heath, M.C., and Hebblethwaite, P.D. 1985. Solar radiation interception by leafless, semileafless and leafed peas (*Pisum sativum* L.) under contrasting field conditions. Ann. Appl. Biol. 107: 309–318. doi:10.1111/j.1744-7348.1985.tb01575.x.

Heath, M.C., and Hebblethwaite, P.D. 1987. Seasonal radiation interception, dry matter production and yield determination for a semileafless pea (*Pisum sativum* L.) breeding selection under contrasting field conditions. Ann. Appl. Biol. 110: 413–420. doi:10.1111/j.1744-7348.1987.tb03273.x.

Helback, H. 1959. Domestication of food plants in the old world: Joint efforts by botanists and archeologists illuminate the obscure history of plant domestication. Science.130:365–372.

Holmgren, P., Jarvis, P.G., and Jarvis, M.S. 1965. Resistances to carbon dioxide and water vapour transfer in leaves of different plant species. Physiol. Plant. 18: 557-573.

Holmes, M.G., and Keiller, D.R. 2002. Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: A comparison of a range of species. Plant, Cell Environ. 25: 85–93. doi:10.1046/j.1365-3040.2002.00779.x.

Hosseinian, F.S., Li, W., and Beta, T. 2008. Measurement of anthocyanins and other phytochemicals in purple wheat. Food Chem. 109: 916–924

Howell, T.A., Musick, J.T., and Talk, J.A., 1986. Canopy temperature of irrigated winter wheat. Trans. ASAE. 29: 1692–1699.

Hsiao, T.C. 1973. Plant Responses to water stress. 1973. Ann Rev. Plant Physiol. 24: 519–570.

Huang, S., Gali, K.K., Tar'an. B., Warkentin, T.D., Bueckert, R.A. 2017. Pea phenology: crop potential in a warming environment. Crop Sci. 57:1540–1551.

Ibrahim, H. M. 2011. Heat stress in food legumes: Evaluation of membrane thermostability methodology and use of infra-red thermometry. Euphytica 180:99–105. doi:10.1007/ s10681-011-0443-9.

Idso, S.B., 1982. Non-water-stressed baseline, a key to measuring and interpreting plant water stress. Agric For Meteorol. 27: 59−70.

Jackson, R.D., Idso, S.B., Reginato, R.J., Pinter, P.J. 1981. Canopy temperature as a crop water stress index. Water. Resour. Res. 17: 1133−1138.

Jacquemoud, S., and Ustin, S.L. 2001. Leaf optical properties: a state of the art. Proceedings of 8th Int. Symp. Physical Measurements and Signatures in Remote Sensing, Aussois, France, pp. 223–232.

Jacquemoud, S., and Baret, F.1990. PROSPECT: a model of leaf optical properties spectra,

Remote Sens. Environ. 34:75−91.

Jefferson, P.G. 1994. Genetic variation for epicuticular wax production in Altai wild rye populations that differ in glaucousness. Crop Sci. 34: 367–371.

Jefferson, P.G., Johnson, D.A., Asay, K.H. 1989. Epicuticular wax production, water status and leaf temperature in *Triticeae* range grasses of contrasting visible glaucousness. Can. J. Plant Sci. 69:513−519.

Jenks, M. a., Rich, P.J., Peters, P.J., Axtell, J.D., and Ashworth, E.N. 1992. Epicuticular Wax Morphology of Bloomless (bm) Mutants in *Sorghum bicolor* L. Int. J. Plant Sci. 153: 311–319 doi:10.1086/297034.

Jenks, M.A., Gaston, C.H., Goodwin, M.S., Keith, J.A., Teusink, R.S., Wood, K.V. 2002. Seasonal variation in cuticular waxes of Hosta genotypes differing in leaf surface glaucousness. Horticultural Science 37: 673–677.

Jenks, M.A., Rich, P.J., Rhodes, D., Acworth, E.N., Axtell, J.D., Chang-Kui Dinga, C. 2000. Leaf sheath cuticular waxes on bloomless and sparse-bloom mutants of *Sorghum bicolor*. Phytochemistry 54: 577–584.

Jensen, M. E., Burman, R. D., Allen, R. G. 1990. Evapotranspiration and irrigation water requirements: A manual. New York, N.Y: The Society.

Jiang, Y., Lahlali, R., Karunakaran, C., Kumar, S., Davis, A.R., and Bueckert, R.A. 2015. Seed set, pollen morphology and pollen surface composition response to heat stress in field pea. Plant. Cell Environ. 38: 2387–2397. doi:10.1111/pce.12589.

Jeuffroy, M., Duthion, C., Meynard, J., and Pigeaire, A. 1990. Effect of a short period of high day temperatures during flowering on the seed number per pod of pea (*Pisum sativum* L.) Agron. 10: 139–145. doi:10.1051/agro:19900207.

Jordan, W.R., Shouse, P.J., Blum, A., Miller, F.R., Monk, R.L. 1984. Environmental physiology of sorghum. II. Epicuticular wax load and cuticular transpiration. Crop Science 24: 1168–1173.

Jones, H.G., and Rotenberg, E. 2011. Energy, radiation and temperature regulation in plants. e LS. John Wiley and Sons Ltd, Chichester. http://www.els.net doi:10.1002/9780470015902.a0003199.pub2.

Karim, M.A., Fracheboud, Y., and Stamp, P. 1999. Photosynthetic activity of developing leaves of Zea mays is less affected by heat stress than that of developed leaves. Physiol. Plant. 105: 685–693. doi:10.1034/j.1399-3054.1999.105413.x.

Karr, E.J., Linck, J., and Swanson, C. 1959. The effect of short periods of high temperature during day and night periods on pea yields. Am. J. Bot. 46: 91–93. doi:10.2307/2439463.

Kashiwagi, J., Krishnamurthy, L., Upadhyaya, H., and Gaur, P. 2008. Rapid screening technique for canopy temperature status and its relevance to drought tolerance improvement in

chickpea. SAT eJournal 6: 1–4.

Kaushal, N., Awasthi, R., Gupta, K., Gaur, P., Siddique, K.H.M., and Nayyar, H. 2013. Heat-stress-induced reproductive failures in chickpea (*Cicer arietinum*) are associated with impaired sucrose metabolism in leaves and anthers. Funct. Plant Biol. 40: 1334–1349 doi:10.1071/FP13082.

Khan, H.R., Paull, J.G., Siddique, K.H.M., and Stoddard, F.L. 2010. Faba bean breeding for drought-affected environments: A physiological and agronomic perspective. F. Crop. Res. 115: 279–286. doi:10.1016/j.fcr.2009.09.003.

Khoo, H. E., Prasad, K. N., Kong, K. W., Jiang, Y., and Ismail, A. 2011. Carotenoids and their isomers: color pigments in fruits and vegetables. Molecules 16: 1710–1738. doi: 10.3390/molecules16021710.

Klepper, B., Browning, V.D., and Taylor, H.M. 1971. Stem diameter in relation to plant water status. Plant Physiol. 48: 683–685. doi:10.1104/pp.48.6.683.

Knipling, E.B. 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. Remote Sens. Environ. 1:155–159.

Kokaly, R.F., and Clark, R.N. 1999. Spectroscopic determination of leaf biochemistry using band-depth analysis of absorption features and stepwise multiple linear regression. Remote Sens. Environ 67: 267–287.

Kosma, D. K., Bourdenx, B., Bernard, A., Parsons, E. P., Lü, S., Joubès, J. 2009. The impact of water deficiency on leaf cuticle lipids of Arabidopsis. Plant Physiol. 151: 1918–1929. doi: 10.1104/pp.109.141911.

Kumar, U., Joshi, A.K., Kumari, M., Paliwal, R., Kumar, S., Röder, M.S. 2010. Identification of QTLs for stay green trait in wheat (*Triticum aestivum* L.) in the 'Chirya 3' x 'Sonalika' population. Euphytica. 174: 437–445.

Kutcher, H.R., Warland, J.S., and Brandt, S.A. 2010. Temperature and precipitation effects on canola yields in Saskatchewan, Canada. Agric. For. Meteorol. 150: 161–165. doi:10.1016/j.agrformet.2009.09.011.

Lambert, R.G., and Linck, A.J. 1958. Effects of high temperature on yield of peas. Plant Physiol. 33: 347–350.

Latowski, D., Kuczynska, P., and Strzalka, K. 2011. Xanthophyll cycle – a mechanism protecting plants against oxidative stress. Redox Rep 16:78–90.

Larkindale, J., Hall, J.D., Knight, M.R., and Vierling, E. 2005. Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol. 138:882–97.

Lesk, C., Rowhani, P., and Ramankutty, N. 2016. Influence of extreme weather disasters on global crop production. Nature 529: 84–87. Nature Publishing Group.

doi:10.1038/nature16467.

Lewis, G.P., Schrire, B., Mackinder, B., and Lock, M. 2005. Legumes of the World. (Richmond, UK: Kew Publishing).

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 148:350–382.

Lobell, D.B., and Field, C.B. 2007. Global scale climate-crop yield relationships and the impacts of recent warming. Environ. Res. Lett. 2. doi:10.1088/1748-9326/2/1/014002.

Lopes, M. S., and Reynolds, M. P. 2012. Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. J. Exp. Bot. 63: 3789–3798. doi: 10.1093/jxb/ers071.

Ludlow, M.M., and Muchow, R.C. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy 43: 107–153.

Ma, B.L., Dwyer, L.M., Costa, C., Cober, E.R., and Morrison, M.J. 2001. Early prediction of soybean yield from canopy reflectance measurements. Agronomy J. 93: 1227–1234.

Macey, M.J.K., Barber, H.N. 1970. Chemical genetics of wax formation on leaves of *Pisum sativum* L. Phytochem. 9: 5–12.

Maestri, E., Klueva, N., Perrotta, C., Gulli, M., Nguyen, H., and Marmiroli, N. 2002. Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Mol. Biol. 48: 667–681. doi:10.1023/A.

Marias, D.E., Meinzer, F.C., and Still, C. 2017. Impacts of leaf age and heat stress duration on photosynthetic gas exchange and foliar non-structural carbohydrates in *Coffea arabica* L. Ecol. Evol. 7: 1297–1310. doi:10.1002/ece3.2681.

Martin, R.J., and Jamieson, P.D. 1996. Effect of timing and intensity of drought on the growth and yield of field peas (*Pisum sativum* L.). New Zeal. J. Crop Hortic. Sci. 24: 167–174. doi:10.1080/01140671.1996.9513949.

Martin, I. J. Tenoria, L., and Ayerbe, L. 1994. Yield, growth and water use of conventional and semi-leafless peas in semi-arid environments. Crop Sci. 34:1576–1583.

McPhee, K. 2003. Dry pea production and breeding: A mini review. Food, Agriculture and Environment. 1: 64-69.

Mekis, É., and Vincent, L.A. 2011. An overview of the second generation adjusted daily precipitation dataset for trend analysis in Canada. Atmos. Ocean 49: 163–177. doi:10.1080/07055900.2011.583910.

Minitab.2018. N.A: Minitab Inc.

Misra, A.N., Latowski, D., Stirzalka, K. 2006. The xanthophyll cycle activity in kidney bean and cabbage leaves under salinity stress. Biomed. Life Sci. 53: 102–109.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11: 15–19. doi:10.1016/j.tplants.2005.11.002.

Mol, J., Grotewold, E., and Koes, R. 1998. How genes paint flowers and seeds. Trends Plant Sci. 3: 212–217.

Murray, J.R., and Hackett, W.P.1991. Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. Plant Physiology 97: 343–351.

Myles S., Peiffer J., Brown P. J., Ersoz E. S., Zhang Z., Costich D. E., Buckler, E.D. 2009. Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell 21: 2194–2202. 10.1105/tpc.109.068437.

Nakano, H., Kobayashi, M., Terauchi, T. 1998. Sensitive stages to heat stress in pod setting of common bean (*Phaseolus vulgaris* L.). Japanese J. Tropical Agric. 42:78–84.

Nash, D, Miyao, M., Murata, N. 1985. Heat inactivation of oxygenevolution in photosystem II particles and its acceleration bychloride depletion and exogenous manganese. Biochim Biophys Acta 807:127–133.

Nelson, J.C. 1988. Genetic associations between photosynthetic characteristics and yield: review of the evidence. Plant Physiol. Biochem. 26: 543–554.

Nicolas, M.E., Gleadow, R.M., and Dalling, M.J. 1984. Effects of drought and high temperature on grain growth in wheat. Aust. J. Plant Physiol. 11: 553. doi:10.1071/PP9840553.

Nonnecke, I., Adedipe, N., and Ormrod, D. 1971. Temperature and humidity effects on the growth and yield of pea cultivars. 51: 479–484.

Ollinger, S.V. 2011. Sources of variability in canopy reflectance and the convergent properties of plants. New Phytologist, 189, 375–394. http://dx.doi.org/10.1111/j.1469-8137.2010.03536.x.

Osborne, S.L., Schepers, J.S., Francis, D.D., and Schlemmer. M.R. 2002. Use of spectral radiance to estimate in-season biomass and grain yield in nitrogen and water-stressed corn. Crop Sci. 42:165–171.

Osório, J., Osório, M.L., and Romano, A. 2012. Reflectance indices as nondestructive indicators of the physiological status of *Ceratonia siliqua* seedlings under varying moisture and temperature regimes. Funct. Plant Biol. doi:10.1071/FP11284.

Othman, R., Mohd Zaifuddin, F.A., and Hassan, N.M. 2014. Carotenoid biosynthesis regulatory mechanisms in plants. J. Oleo Sci. 63: 753–760.

Peñuelas, J., Munné-Bosch, S., Llusià, J., and Filella, I. 2004. Leaf reflectance and photo- and antioxidant protection in field-grown summer-stressed *Phillyrea angustifolia*. Optical signals of oxidative stress? New Phytol. 162:115–124. doi:10.1046/j.1469-8137.2004.01007.x.

Penuelas, J., Pinol, J., Ogaya, R., and Filella, I. 1997. Estimation of plant water concentration by the reflectance Water Index WI (R900/R970). Int. J. Remote Sens. 18: 2869–2875. doi:10.1080/014311697217396.

Peñuelas J. Fillella I. Biel C. Serrano L. Save R.1993. The reflectance at the 950-970 region as an indicator of plant water stress. Int. J. Remote Sens. 14: 1887–1905.

Peñuelas J. Gamon J. A. Fredeen A. L. Merino J. Field C. B.1994. Reflectance indices associated with physiological changes in nitrogen and water-limited sunflower leaves. Remote Sens. Environ. 48: 135–146.

Plumley, F.G. Schmidt, G.W. 1995. Light-harvesting chlorophyll a/b complexes: interdependent pigment synthesis and protein assembly. Plant Cell 7: 689–704.

Poggio, S.L., Satorre, E.H., Dethiou, S., and Gonzalo, G.M. 2005. Pod and seed numbers as a function of photothermal quotient during the seed set period of field pea (*Pisum sativum* L.) crops. Eur. J. Agron. 22: 55–69. doi:10.1016/j.eja.2003.12.003.

Porcar-Castell,A., Garcia-Plazaola, J.I., Nichol, C.J., Kolari, P., Olascoaga, B., Kuusinen, N., Fernández-Marín, B., Pulkkinen, M., Juurola, E., Nikinmaa, E. 2012. Physiology of the seasonal relationship between the photochemical reflectance index and photosynthetic light use efficiency. Oecologia 170:313–323.

Porter, J.R, and Gawith, M. 1999. Temperatures and the growth and development of wheat: a review. Eur J Agron 10:23–36.

Powles, S.B. 1984. Photoinhibition of photosynthesis induced by visible light. Annu. Rev. Plant Physiol. 35: 15–44.

Prasad, P.V.V., Boote, K.J., Allen, L.H., and Thomas, M.G. 2002. Effects of elevated temperature and carbon dioxide on seed-set and yield of Kidney Bean (*Phaseolus vulgaris* L.). Global Change Biol. 8: 710–721.

Prasad, P.V.V., Pisipati, S.R., Momčilović, I., and Ristic, Z. 2011. Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. J. Agron. Crop Sci. 197: 430–441. doi:10.1111/j.1439-037X.2011.00477.x.

Prasad, P.V.V., Staggenborg, S. a., and Ristic, Z. 2008. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Advances in Agricultural Systems Modeling Series. 1. 301-355.

Prasad, P.V.V., Bheemanahalli, R., and Jagadish, S.V.K. 2017. Field crops and the fear of heat stress-Opportunities, challenges and future directions. doi:10.1016/j.fcr.2016.09.024.

Premachandra, G.S., Saneoka, H., Fujita, K., Ogata, S. 1992. Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in Sorghum. J. Exp. Bot. 43: 1569–1576.

Price, J.C., and Bausch, W.C. 1995. Leaf area index estimation from visible and near-infrared refl ectance data. Remote Sens. Environ. 52:55–65.

Pumphrey, F. V, and Ramig, R.E. 1990. Field response of peas to excess heat during the reproductive stage of growth. J. Am. Soc. Hortic. Sci. 115: 898–900.

Raison, J.K, Roberts, J.K.M., and Berry, J.A. 1982. Correlation between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of the higher plant, Nerium oleander, to growth temperature. Biochim. Biophys. Acta 688:218–228.

Rayne, S., and Forest, K. 2012. Temperature and precipitation trends in southwestern Saskatchewan tell a complex long-term climate change story. J. Geophys. Res. Atmos. 117: 3–6. doi:10.1029/2012JD017859.

Reicosky, D.C., Deaton, D.E., and Parsons, J.E. 1980. Canopy air temperatures and evapotranspiration from irrigated and stressed soybeans. Agric. Meteorol. 21: 21–35. doi:10.1016/0002-1571(80)90066-7.

Reichler, S.A., Torres, J., Rivera, A.L., Cintolesi, V.A., Clark, G., and Roux, S.J. 2009. Intersection of two signalling pathways: Extracellular nucleotides regulate pollen germination and pollen tube growth via nitric oxide. J. Exp. Bot. 60: 2129–2138. doi:10.1093/jxb/erp091.

Reynolds, M.P., Balota, M., Delgado,M.I.B., Amani, I., Fischer, R.A. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust. J. Plant Physiol. 21: 717–730.

Reynolds, M. P., Ortiz, M. R. J. I., and McNab. 2001. Application of physiology in wheat breeding, CIMMYT, Mexico.

Reynolds, M.P., Manes, Y., Izanloo, A., and Langridge, P. 2009. Phenotyping approaches for physiological breeding and gene discovery in wheat. Ann. Appl. Biol. 155: 309–20.

Richards, R. A., Rawson, H. M., and Johnson, D. A. 1986. Glaucousness in wheat: its develoomentand effect on water use efficiency, gas exchange and photosynthetic tissue temperatures. Aust. J. Plant Physiol. 13: 465–473.

Ridge, P.E., and Pye, D.L. 1985. The effects of temperature and frost at flowering on the yield of peas grown in a mediterranean environment. Field Crops Res. 12: 339–346.

Rizhsky, L., Liang, H., and Mittler, R. 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol. 130: 1143–1151. doi:10.1104/pp.006858.

Rollins, J.A., Habte, E., Templer, S.E., Colby, T., Schmidt, J., and Von Korff, M. 2013. Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (*Hordeum vulgare* L.). J. Exp. Bot. 64: 3201–3212.

doi:10.1093/jxb/ert158.

Rouse, J.W., Haas, R.H., Schell, J.A., and Deering, D.W. 1974. Monitoring vegetation systems in the Great Plains with ERTS, In: S.C. Freden, E.P. Mercanti, and M. Becker (eds) Third Earth Resources Technology Satellite–1 Symposium. Volume I: Technical Presentations, NASA SP-351, NASA, Washington, D.C., pp. 309–317.

Royo, C., N. Aparicio, D. Villegas, J. Casadesus, P. Monneveux, and J.L. Araus. 2003. Usefulness of spectral reflectance indices as durum wheat yield predictors under contrasting Mediterranean conditions. Int. J. Remote Sens. 24:4403–4419.

Sadras, V.O., Lake, L., Chenu, K., McMurray, L.S., and Leonforte, A. 2012. Water and thermal regimes for field pea in Australia and their implications for breeding. Crop Pasture Sci. 63: 33–44. doi:10.1071/CP11321.

Sadras, V.O., Lake, L., Leonforte, A., McMurray, L.S., and Paull, J.G. 2013. Screening field pea for adaptation to water and heat stress: Associations between yield, crop growth rate and seed abortion. Field Crops. Res. 150: 63–73. doi:10.1016/j.fcr.2013.05.023.

Sánchez, F.J., Manzanares, M., De Andrés, E.F., Tenorio, J.L., and Ayerbe, L. 2001. Residual transpiration rate, epicuticular wax load and leaf colour of pea plants in drought conditions. Influence on harvest index and canopy temperature. Eur. J. Agron. 15: 57–70. doi:10.1016/S1161-0301(01)00094-6.

Sangwan, V., and Dhindsa, R.S. 2002. In vivo and in vitro activation of temperature-responsive lant map kinases. Eur. Biochem. Sci. Letters 531: 561–564.

Sehgal, A., Sita, K., Kumar, J., Kumar, S., Singh, S., Siddique, K.H.M., and Nayyar, H. 2017. Effects of Drought, Heat and Their Interaction on the Growth, Yield and Photosynthetic Function of Lentil (*Lens culinaris*) Genotypes Varying in Heat and Drought Sensitivity. Front. Plant Sci. 8: 1776. doi:10.3389/fpls.2017.01776.

Shepherd, T., and Wynne Griffiths, D. 2006. The effects of stress on plant cuticular waxes. New Phytol. 171: 469–499. doi: 10.1111/j.1469-8137.2006. 01826.x

Sims, D.A., and Gamon, J.A. 2003. Estimation of vegetation water content and photosynthetic tissue area from spectral reflectance: A comparison of indices based on liquid water and chlorophyll absorption features. Remote Sensing of Environ. 84: 526–537.

Singh, A., Selvi, M.T., Sharma, R. 1999. Sunlight-induced anthocyanin pigmentation in maize vegetative tissues. J. of Exp. Botany 50: 1619–1625.

Sita, K., Sehgal, A., HanumanthaRao, B., Nair, R.M., Vara Prasad, P. V., Kumar, S., Gaur, P.M., Farroq, M., Siddique, K.H.M., Varshney, R.K., and Nayyar, H. 2017. Food legumes and rising temperatures: effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. Front. Plant Sci. 8: 1658 doi:10.3389/fpls.2017.01658.

Stanfield, B., Ormord, D.P., and Fletcher, H.F. 1966. Response of peas to environment II, effects of temperature in controlled environment cabinets. Can. J. Plant Sci. 46: 195–203.

Steyn, W.J., Wand, S.J.E., Holcroft, D.M., and Jacobs, G. 2002. Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. New Phytol. 155: 349–361. doi:10.1046/j.1469-8137.2002.00482.x.

Smýkal, P., Aubert, G., Burstin, J., Coyne, C. J., Ellis, N. T. H., Flavell, A. J., Ford, R., Hybl, M., Macas, J., Neumann, P., McPhee, K., Redden, R., Rubiales, D., Weller, J., and Warkentin, T. 2012. Pea (*Pisum sativum* L.) in the genomic era. Agronomy 2: 74–115. doi: 0.3390/agronomy2020074.

Snoad, B. 1974. A preliminary assessment of "leafless peas." Euphytica 23: 257–265. doi:10.1007/BF00035866.

Sun, P., Grignetti, A., Liu, S., Casacchia, R., Salvatori, R., Pietrini, F., Loreto, F., and Centritto, M. 2008. Associated changes in physiological parameters and spectral reflectance indices in olive (*Olea europaea* L.) leaves in response to different levels of water stress. Int. J. Remote Sens. 29:1725–43. https://doi.org/ 10.1080/01431160701373754

Tebaldi, C., Hayhoe, K., Arblaster, J.M., and Meehl, G.A. 2006. Going to the extremes: An intercomparison of model-simulated historical and future changes in extreme events. Climate Change: 79: 185–211, doi: 10.1007/s10584-006-9051-4.

Thomas, H. and Smart, C.M. 1993. Crops that stay green. Ann. Appl. Biol. 123:193–201.

Tripathy, B.C., and Oelmuller, R. 2012. Reactive oxygen species generation and signalling in plants. Plant Signaling and Behavior 7: 1621–1633.

Tzitzikas, E. N., Vincken, J.P., Groot, J., De Gruppen, H., and Visser, R.G.F. 2006. Genetic variation in pea seed globulin composition. J. Agric. Food. Chem. 54: 425–433.

Wahid, A., Gelani, S., Ashraf, M., and Foolad, M.R. 2007. Heat tolerance in plants: An overview. Environ. Exp. Bot. 61: 199–223. doi:10.1016/j.envexpbot.2007.05.011.

Wang, J., Gan, Y.T., Clarke, F., and McDonald, C.L. 2006. Response of chickpea yield to high temperature stress during reproductive development. Crop Sci. 46: 2171–2178. doi:10.2135/cropsci2006.02.0092.

Warkentin T. D., Smykal P., Coyne C. J., Weeden N., Domoney, C., Bing, D., Leonforte, A., Xuxiao, Z., Dixit, G.P., Boros, L., McPhee, K.E., McGee, R.J., Burstin, J., and Ellis, T.H.E. 2015. Pea (*Pisum sativum* L.), in grain legumes, Series Handbook of Plant Breeding, ed De Ron A. M., editor. (New York, NY: Springer Science and Business Media), 37–83.Wilson, D.R.

Warren, G.F. 1998. Spectacular increases in crop yields in the twentieth century. Weed Technol. 12: 752–760.

Watanabe, N., Naruse, J., Austin, R.B., and Morgan, C.L. 1995. Variation in thylakoid proteins

and photosynthesis in Syrian landraces of barley. Euphytica 82: 213–220.

Wiegand, C.L., Richardson, A.J., Escobar, D.E., and Gerbermann, A.H. 1991.Vegetation indices in crop assessments. Remote Sens. Environ. 35: 105–119.

Weiss, D. 2000. Regulation of flower pigmentation and growth: multiple signalling pathways control anthocyanin synthesis in expanding petals. Physiologia Plantarum 110: 152-157.

Welch, R. W., and Grifliths, D. W. 1984. Variation in the oil content and fatty acid composition of field beans (*Vici faba*) and peas (*Pisum* spp.) J. Sci. Food Agric. 35: 1282–1289.

Wiegand, C. L., and Richardson, A. J. 1990. Use of spectral vegetation indices to infer leaf rea, evapotranspiration and yield: I. Rationale. Agron. J. 82:623–629.

Willick, I.R., Lahlali, R., Vijayan, P., Muir, D., Karunakaran, C., and Tanino, K.K. 2017. Wheat flag leaf epicuticular wax morphology and composition in response to moderate drought stress are revealed by SEM, FTIR-ATR and synchrotron X-ray spectroscopy. Physiologia Plantarum 62: 316–332.

Xu, W., Rosenow, D.T., and Nguyen, H.T. 2000. Stay green trait in grain sorghum: Relationship between visual rating and leaf chlorophyll concentration. Plant Breed. 119: 365–367. doi:10.1046/j.1439-0523.2000.00506.x.

Xue, J., and Su, B. 2017. Significant remote sensing vegetation indices: A review of developments and applications. Journal of Sensors. doi:10.1155/2017/1353691.

Yamasaki, H., Uefuji, H., and Sakihama, Y. 1996. Bleaching of the red anthocyanin induced by superoxide radical. Arch. Biochem. Biophys. 332: 183-186.

Yamasaki, H. 1997. A function of colour. Trends in Plant Sci. 2: 7-8.

Yoo, C.Y., Pence, H.E., Hasegawa, P.M., and Mickelbart, M.V.2009. Regulation of transpiration to improve crop water use. Crit. Rev. Plant Sci. 28: 410–431.

Yoshida, H., Tomiyama, Y., Tanaka M., Mizushina Y. 2007. Characteristic profiles of lipid classes, fatty acids and triacylglycerol molecular species of peas (*Pisum sativum* L.). Eur. J. Lipid Sci. Tech. 109: 600–607.

Zarate-Valdez, J.L., Whiting, M.L., Lampinen, B.D., Metcalf, S., Ustin, S.L., and Brown, P.H. 2012. Prediction of leaf area index in almonds by vegetation indexes. Comput. Electron. Agric. 85: 24–32. doi:10.1016/j.compag.2012.03.009.

Zhang, C., Tar'an, B., Warkentin, T., Tullu, A., Bett, K.E., Vandenberg, B., and Somers, D.J. 2006. Selection for lodging resistance in early generations of field pea by molecular markers. Crop Sci. 46: 321–329. doi:10.2135/cropsci2005.0123.

Zohary, D., and M. Hopf. 2002. Domestication of plants in the old world: The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Third Edition. Oxford University Press Inc. New York.

APPENDIX A

Leaf wax extraction and determination protocol

Wax extraction and quantification was done according to Sanchez et al (2001), originally from Ebercon et al (1977).

Leaf sample

- Use fully expanded mature leaf sample from second or third node
- Separately scan the stalk (petiole) and leaf surfaces (lamina) and determine the projected areas

Chemicals

- Chloroform ($CHCl_3$)
- Sulfuric acid (H_2SO4)
- Potassium dichromate ($K_2\ Cr_2\ O_7$)
- Deionized water (H_2O)

Reagent preparation

- 40 ml di H_2O, 20g, $K_2\ Cr_2\ O_7$, heat and slurry mixed,
- Make up to 1 L by adding 100% H_2SO4 while stirring, and keep on heat and stirring until clear solution appears

Wax extraction and determination

- 10 ml Chloroform in test tube, dip the leaf sample into the chloroform for 15 sec one a time
- Filter evaporate the chloroform at 35 ºC water bath
- Add 5 ml reagent and keep the tubes in boiling water for 30 min
- Cool, and add 5 ml di water (10 total)
- Standards prepared using serious of known concentrations of beeswax
- Read spectroscopic absorbance at 590 nm

APPENDIX B

Chlorophyll and carotenoid extraction and determination

Chlorophyll measurement was performed according to Lichtenthaler's (1987)

Plant materials

- Use fully expanded mature leaf sample from second or third node
- Separately scan the stalk (petiole) and leaf surfaces (lamina) and determine the projected areas
- For the leaf surface chlorophyll and carotenoid extraction 1.22 cm^2 stipule disc was used
- For the petiole chlorophyll and carotenoid extraction area was determined by winRhizo root area scanner

Chemical

- 100% Acetone

Extraction and quantification

- The samples were placed in a 5 ml glass vials with a tight cap and 3 ml of 100% acetone was added and kept for 6 hours at room temperature for complete extraction.
- Then, the samples were homogenized by vortex and centrifuged for 5 min at 5000 rpm.
- The clear supernatant solution was used for absorbance measurement using the spectrophotometer at wavelengths of 470, 645, 662 and 710 nm.
- Concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoid were determined using the following equations:

Chlorophyll a ($\mu g ml^{-1}$) = $[11.24(A662-A_{710})]-[2.04(A645-A_{710})]$

Chlorophyll b ($\mu g ml^{-1}$) = $[20.13(A645-A_{710})]-[4.19(A662-A_{710})]$

Total Chlorophyll ($\mu g ml^{-1}$) = $[7.05(A662-A_{710})] + [18.09(A645-A_{710})]$

Total carotenoid ($\mu g ml^{-1}$) = $[1000(A470-A_{710})]-[1.90$ Chlorophyll a$]-[63.14$ Chlorophyll b$]$

APPENDIX C

Anthocyanin extraction and determination

Anthocyanin quantification was performed using spectrophotometric method according to Abdel-Aal and Hucl (1999)

Plant materials

- Use fully expanded mature leaf sample from second or third node
- Separately scan the stalk (petiole) and leaf surfaces (lamina) and determine the projected areas
- For the leaf surface chlorophyll and carotenoid extraction 1.33 cm^2 stipule disc was used
- For the petiole chlorophyll and carotenoid extraction area was determined using winRHIZO root area scanner

Chemicals

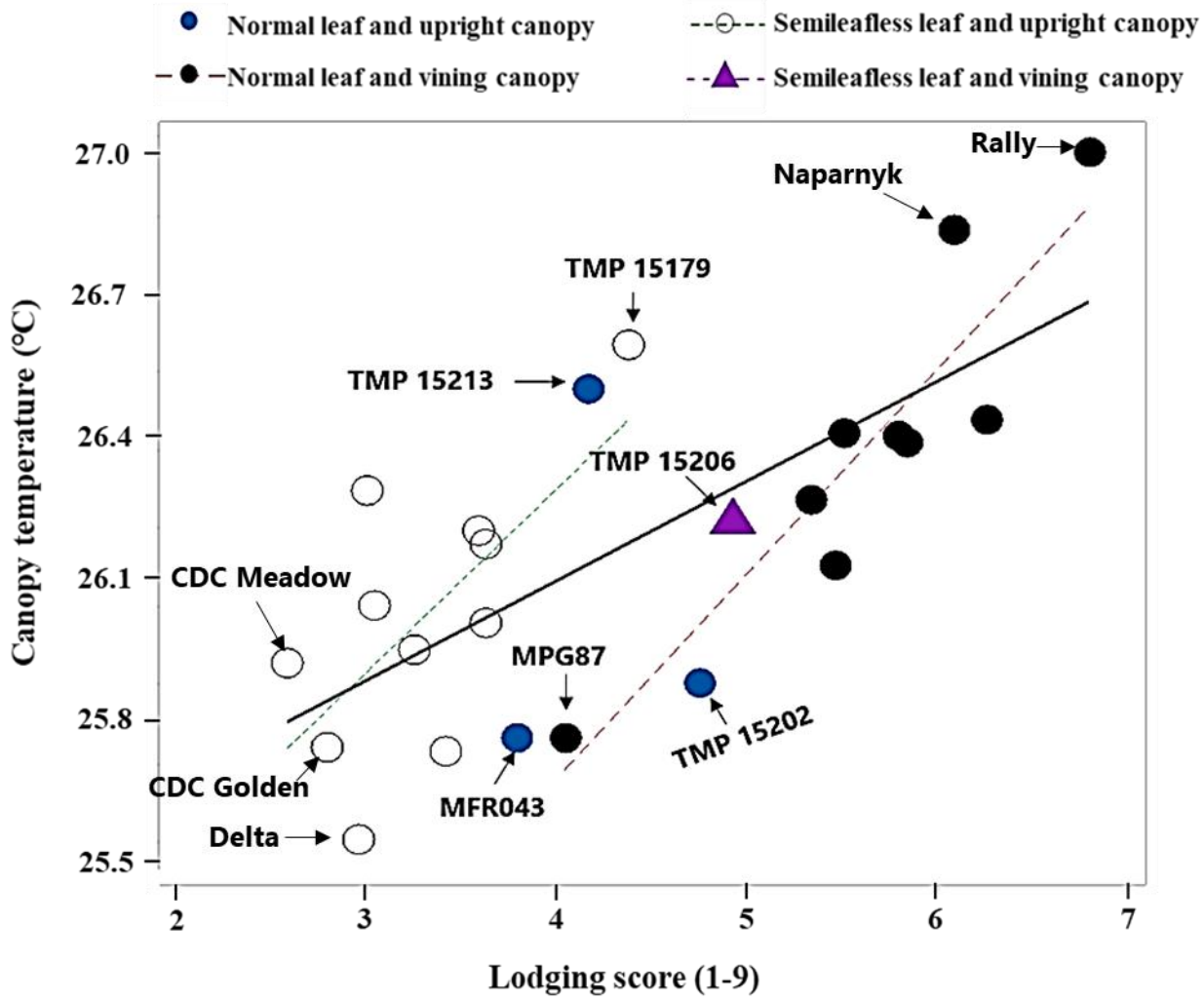
- 95% Ethanol (C_2H_6O)
- Hydrochloric acid (HCl)
- di water

Extraction and quantification

- Acidified ethanol (pH1, 85:15 volume/volume ratio of 95% ethanol and 1.428N HCl) was used as the extraction reagent
- The lamina and petiole samples were separately placed in 5 ml glass vials and 3 ml of acidified ethanol was added
- Kept in the solution overnight at room temperature for complete extraction.
- Then, the samples were mixed by vortex and centrifuged for 5 min at 5000 rpm.
- The clear supernatant solution was used for absorbance reading at 535 and 663 nm using the spectrophotometer.
- Total anthocyanin concentrations in µg cm^{-2} was calculated as A535-0.24(A663) (Murray and Hackett, 1991).
- The 535 nm is the wavelength peak for anthocyanin absorbance under the above extraction conditions.

APPENDIX D

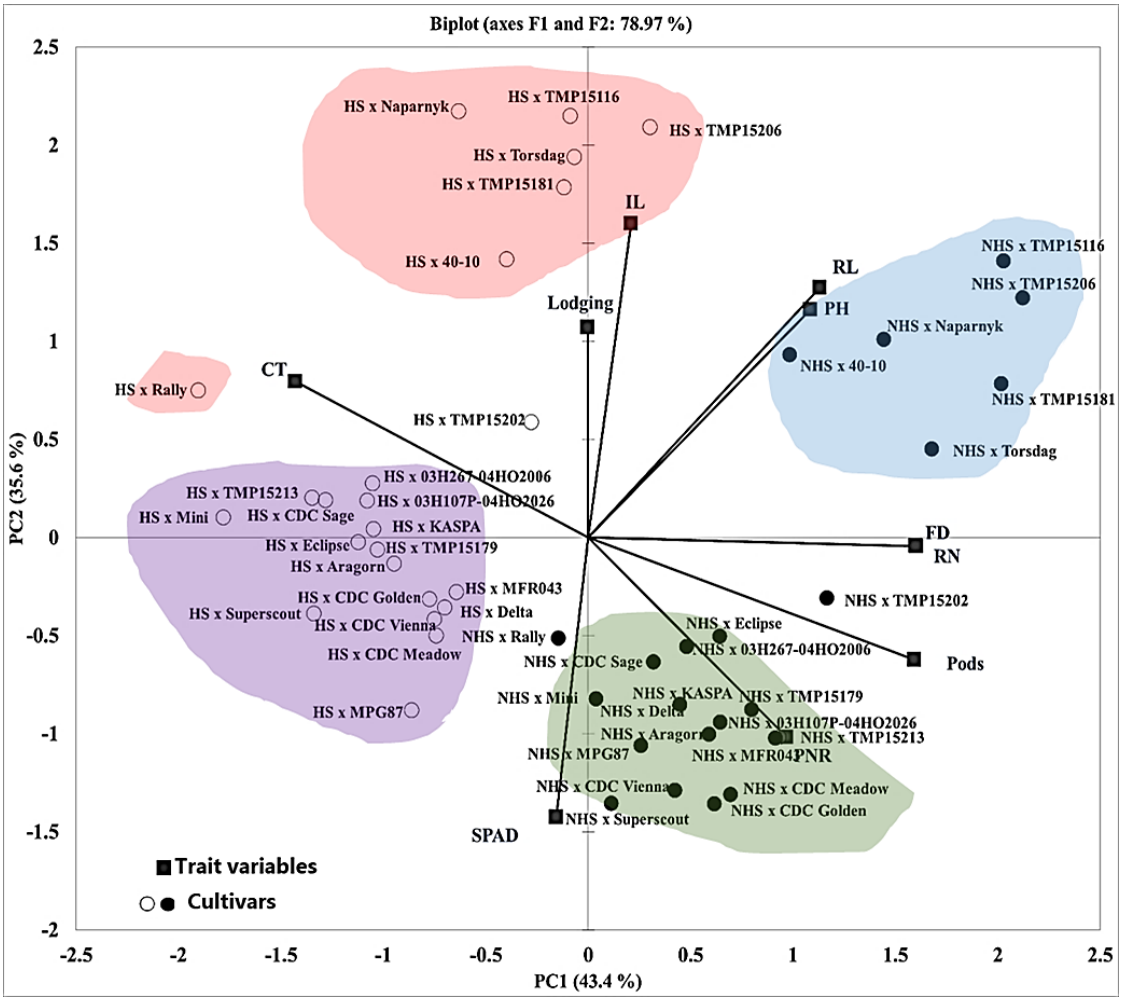
Lodging contributed to high canopy temperature



Lodging and canopy temperature correlation. MFR043 had a normal leaf type with an upright nature likely due to strong stem, and thus had low lodging score and canopy temperature. MPG87 had normal and vine leaf but with lower lodging score, and thus low canopy temperature. These cultivar also had high wax concentration which generally correlates negatively with canopy temperature. Each symbol is averaged over 4 reps and 6 environments.

APPENDIX E

Principal component analysis



Bi-plot from principal components demonstrating relationship between number of reproductive nodes, pod number, pod to node ratio, chlorophyll content estimation by SPAD meter (SPAD), canopy temperature, lodging score, reproductive stem length, internode length, plant height, and flowering duration pea cultivars grown under heat stressed (Saskatoon 2015 late seeding date, empty circles) and non-heat stressed (Saskatoon 2016 normal seeding date, full circles) environments. The PCA showed four major clear clustering among the 24 cultivars under the two environments, and the clustering was primarily caused by the leaf type and canopy habit response to the heat stress. Eigenvalue proportions of the first two components are marked as a percentage on the axis label.
HS: Heat stress, NHS: non-heat stress (control environment SN16). The name of each cultivar is mentioned near their corresponding symbols. Each symbol is averaged over 4 reps.